

## ESHG PLENARY LECTURES

### PL1.1

#### Understanding the Influence of Conditional Host-Karyome-Microbiome Interactions in Health and Disease

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Post-genomic technologies are being widely applied to improve the understanding of adverse drug reactions and the molecular basis of human disease. Metabonomics is an approach that enables multivariate profiling of the integrated metabolic responses of complex systems to patho-physiological stress, and so involves understanding the way the whole metabolic regulatory system varies with interventions thus providing complementary information to genomics and proteomics. Mammalian biochemistry is strongly influenced by the host karyome (genome) and gut microbiome symbionts that can alter drug metabolism and toxicity; the study of these transgenomic interactions is termed "global systems biology". Because these interactions are mediated by a large number of co-metabolic processes the system state integrity can be evaluated via metabolic profiling of biofluids. With the growing desire to apply systems biology tools to understanding human disease processes at the population level where massive cohorts need to be investigated, it is necessary to use analytical and statistical methods that report on *whole system state* non-invasively, hence the attraction of biofluid analyses described here.

NMR spectroscopy and chromatographic linked MS methods have been applied to characterize and quantify a wide range of metabolites in biological fluids and tissues to explore the biochemical consequences of drug-induced toxicity and human disease. In disease or toxicity states metabolic profiles and NMR and mass spectra are changed characteristically in different toxicity or disease conditions according to the exact site and mechanism of the lesion. The use of chemometrics allows interrogation of spectroscopic data and can give direct diagnostic information and aid the detection of novel biomarkers of disease and the integration of metabolic data with other omics sets. Such diagnostics can be extremely sensitive for the detection of low level damage in a variety of organ systems and is potentially a powerful new adjunct to conventional procedures for disease assessment and can help explain environment-gene interactions that give rise to idiosyncratic toxicity of drugs in man. Examples of the application of metabonomics to personalised healthcare (1) and population screening to detect new "Metabolome-Wide Associations" with disease risk factors (2).

1. Clayton, T.A. Nicholson, J.K. et al (2006) Pharmaco-metabonomic phenotyping and personalised drug treatment. *Nature* 440 (20) 1073-1077.
2. Holmes, E. Nicholson, J.K. et al (2008) Human Metabolic Phenotype Diversity and its Association with Diet and Blood Pressure. *Nature* (in press).

### PL1.2

#### Cell competition, apoptosis and tumour progression in *Drosophila*

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Larvae homozygous for mutations at the tumour suppressor genes of *Drosophila* lethal giant larvae (lgl), scribble (scribb) or disc large (dlg) develop extensive neoplastic tumours that affect principally the central nervous system and the imaginal discs. Thus the lack of any of these products is sufficient to transform normal imaginal cells into tumorous cells. However, imaginal cells mutant for any of these genes are unable to develop a tumour if they are surrounded by non-tumour cells. We have been studying the behaviour of clones of lgl mutant cells in the wing disc to study the interactions between tumour and non-tumour cells. We find that as a rule lgl clones are eliminated from the wing disc by a process akin to cell competition: they enter apoptosis mediated by the JNK pathway and the interactions leading to the disappearance of the mutant cells take place at the border of the clones. We have also found that when these cells contain an additional factor conferring a high proliferation rate, the lgl mutant cells are transformed into "supercompetitors", which are able to eliminate surrounding non-

tumour cells and give rise to invasive neoplastic tumours that colonise the entire disc

### PL1.3

#### Human evolution: palaeontology and ancient DNA

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### PL2.1

#### Mutations in the pericentrin (PCNT) gene cause primordial dwarfism

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The growth of an individual grossly depends on regulation of cell size and cell division and dysfunction of the pathways involved not only results in somatic undergrowth but contributes to a wide variety of pathological conditions.

Using positional cloning, we found in a total of 25 patients that biallelic loss-of-function mutations in the pericentrin (PCNT) gene cause microcephalic osteodysplastic primordial dwarfism type Majewski II (MOPD II, MIM 210720). Adults with this rare inherited condition belong to the shortest of the short having a height of about 100 centimeters and a brain size comparable to that of a three-month old baby, but are of near-normal intelligence. Truncal obesity, type 2 diabetes and a high risk of stroke have been noted in older individuals with MOPD II.

PCNT is known to mediate nucleation of microtubules by anchoring the  $\gamma$ -tubulin ring complex, thus initiating the assembly of the mitotic spindle apparatus. We show that PCNT mutations cause absence of the protein resulting in disorganized mitotic spindles, premature sister chromatid separation and missegregation of chromosomes in patient cells. Our findings thus characterize MOPD II as a distinct clinical entity linking a key protein of the centrosome to dwarfism and a high risk of diabetes and stroke.

Similarities between MOPD II individuals and the Late Pleistocene hominid fossils from the island of Flores, Indonesia, also known as "hobbits", suggest that these do not represent a diminutive, small-brained new species, *Homo floresiensis*, but are pathological modern humans.

Rauch et al. 2008, Science Feb 8, 319:816-9.

### PL2.2

#### Meta-analysis of genome-wide association data and large-scale replication identifies several additional susceptibility loci for type 2 diabetes

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Genome-wide association (GWA) studies have identified multiple new loci at which common variants modestly but reproducibly contribute to risk of type 2 diabetes (T2D). However, established variants, common and rare, explain only a small proportion of the heritability of T2D. To increase the power to discover alleles of modest effect, we performed

meta-analysis of three T2D GWA scans (DGI, FUSION, WTCCC) encompassing 10,128 European-descent individuals (4549 cases and 5579 controls) and ~2.2 million SNPs (directly genotyped and imputed). Even after excluding known type 2 diabetes loci, we saw a strong enrichment of highly associated variants (426 with  $p < 10^{-4}$  vs. 217 expected by chance). Sixty nine SNPs were taken forward to an initial round of replication in up to 22,426 individuals, and 11 SNPs with replication  $p < 0.005$  were tested in additional individuals from the deCODE, KORA, Danish and HUNT T2D studies with an effective sample size of up to 18,066. Multiple new loci with strong evidence for association were observed, including the *JAZF1* (OR[95%CI]: 1.11[1.08-1.14],  $p = 8.8 \times 10^{-12}$ ), *CDC123/CAMK1D* (OR1.10[1.07-1.14],  $p = 5.6 \times 10^{-9}$ ), *ADAMTS9* (OR1.10[1.07-1.14],  $p = 9.5 \times 10^{-9}$ ), and *THADA* (OR 1.17[1.11-1.23],  $p = 4.5 \times 10^{-8}$ ) gene regions. These loci provide additional biological clues about the inherited basis of T2D, and the relatively small effects point to the need for large discovery and follow-up samples.

#### PL2.3

#### G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth

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Hypotrichosis simplex (MIM 146520 and MIM 605389) is a group of hereditary non-syndromic human alopecias that affects men and women equally. The hair loss is diffuse and progressive, and usually begins in early childhood. We mapped an autosomal recessive form of this disorder to chromosome 13q14.11-13q21.33, and identified homozygous truncating mutations in *P2RY5*, a gene which encodes an orphan G protein-coupled receptor. We analysed expression-patterns of *P2RY5* in various human and mouse tissues and performed western blot and immunofluorescence analyses to characterize the protein. Furthermore, we identified oleoyl-L-α-lysophosphatidic acid (LPA), a bioactive lipid, as being a ligand for P2Y5 in reporter gene and radioligand binding experiments. Homology and studies of signalling transduction pathways suggest that P2Y5 is a member of a subgroup of LPA receptors, which also includes LPA4 and LPA5. As *P2RY5* is expressed in human hair follicle cells, but LPA4 and LPA5 are not, a loss of P2Y5 function will not be compensated for, and will ultimately lead to pathological changes and hair loss.

Our study is the first to implicate a G protein-coupled receptor as being essential for and specific to the maintenance of human hair growth. With the functional characterization of the P2Y5 receptor, we identify the missing link which is required for the transmission of the LPA signal through the cell membrane in hair follicle cells. This finding may provide opportunities for new therapeutic approaches to the treatment of hair loss in humans.

#### PL2.4

#### X-linked protocadherin 19 mutations cause female-limited epilepsy and cognitive impairment

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EFMR (Epilepsy and Mental Retardation limited to Females) is a disorder with an X-linked mode of inheritance and unusual expression pattern. Disorders arising from mutations on the X chromosome are typically characterized by affected males and unaffected carrier females. In contrast, EFMR spares transmitting males and affects only carrier females. Unraveling the biology of this conundrum depends upon the identification of the molecular defect underlying EFMR. Aided by systematic re-sequencing of 737 X chromosome genes we identified different protocadherin 19 (*PCDH19*) gene mutations in seven families. Five mutations result in the introduction of a premature termination codon. Study of two of these demonstrated nonsense mediated decay of *PCDH19* mRNA. The two missense mutations are predicted to affect adhesiveness of *PCDH19* through impaired calcium binding. *PCDH19* is expressed in human and mouse developing brain and is the first member of the cadherin superfamily to be directly implicated in epilepsy or mental retardation. To explain the reversed, sex-limited expression pattern for EFMR, we propose rescue in males by a human specific Y chromosome gene protocadherin 11Y (*PCDH11Y*).

#### References:

Ryan SG et al. Epilepsy and mental retardation limited to females: an X-linked dominant disorder with male sparing. *Nat Genet.* 1997 Sep;17(1):92-5.

Scheffer IE et al. Epilepsy and mental retardation limited to females: an under-recognized disorder. *Brain.* 2008 Jan 29; [Epub ahead of print].

#### PL2.5

#### Genomic structural variation profiles of world human populations

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<sup>1</sup>Center for Genomic Regulation (CRG), Barcelona, Catalonia, Spain, <sup>2</sup>Pompeu Fabra University (UPF), Barcelona, Catalonia, Spain, <sup>3</sup>Public Health and Epidemiology Network Biomedical Research Center (CIBERESP), Barcelona, Catalonia, Spain, <sup>4</sup>National Genotyping Center (CeGen), Barcelona, Catalonia, Spain. Genomic variants can contribute to genetic disease, and are potential substrates for natural selection resulting in phenotypic differences between individuals. The use of genome-wide molecular methods have revealed the existence of Copy Number Variants (CNVs), genomic segments ranging in size from one kb to several megabases, that are present at variable copy number in comparison with a reference genome. The aim of our study was to determine the existence of population-specific genomic structural variation and to identify genes located in these regions that might contribute to phenotypic differences as well as to differential susceptibility to common disease and environmental exposures of human populations. We have selected 343 individuals from 11 populations from the HGDP-CEPH panel (Biaka- Mbuti Pygmy, Bantu, Mozabite, Bedouin, Brahim, Hazara, Yakut, Papuan-Melanesian, French, Pima and Maya) and 134 individuals from the three populations of the HapMap collection (YRI, CHB and CEU). To detect structural variation we have used array-CGH (Agilent 244K) and array-based comparative genome intensity (Illumina). We have observed differences between populations in 179 loci. 122 of these were already described in the Database of Genomic Variants and 58 coincide with segmental duplications. Interestingly, a number of genes involved in different common disorders or to have phenotypic differences between population groups were found to be variable in copy number among human populations (i.e. RHD, CFHR1, CFHR3 and PRSS1). These loci and others could explain differences in disease predisposition among individuals from different populations and could provide important clues on the adaptation of humans to different environments.

#### PL2.6

#### Acetylcholine receptor pathway mutations explain various fetal akinesia deformation sequence disorders

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Impaired fetal movement causes growth retardation, fetal hydrops, polyhydramnios, pulmonary hypoplasia, multiple joint contractures with or without webbing (pterygia), and other features, summarized under the term fetal akinesia deformation sequence (FADS).

We sequenced 75 patients with severe FADS and found mutations in the  $\gamma$ ,  $\alpha 1$  and  $\delta$  subunit of the AChR as well as in the receptor associated protein of the synapse, rapsyn.

Our major conclusions are that

- FADS is a frequent genetic condition.
- Doctors in pre- and neonatal care are often confronted with FADS but are not as aware of possible involvement of AChR pathway mutations as a neurologist would be.
- More awareness of the potential involvement of the AChR pathway will save the lives of some newborns.

Our findings are important to the understanding of cellular biology, genetics and clinical relevance of AChR mutations especially in pre- and neonatal care. Early neonatal diagnosis in one of our families helped to start effective treatment for the second affected child, whereas the first affected - undiagnosed - died in infancy. One major conclusion is that the inclusion of clinical and molecular data from prenatally deceased siblings is crucial to elucidate severe phenotypes of AChR pathway mutations.

We suggest that the AChR pathway contributes to a broad spectrum of intrauterine phenotypes and should be examined functionally and genetically in patients with recurrent spontaneous abortions, fetal akinesia, hydrops, pterygia, or inborn contractures.

#### PL2.7

##### Plastin 3 protects against spinal muscular atrophy (SMA) - the first report of a fully protective modifier of a Mendelian disease

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Homozygous deletion of *SMN1* leads to spinal muscular atrophy (SMA), the most frequent genetic cause of early childhood lethality. In rare instances, however, individuals carrying the same *SMN1* mutations as their affected siblings are found to be fully asymptomatic, thereby suggesting the action of modifier genes. By comparing the transcriptomes of lymphoblastoid cell lines from unaffected and affected *SMN1*-deleted siblings, we identified plastin 3 (*PLS3*, localized on Xq23) to be abundant in unaffected, but not affected counterparts. We showed that *PLS3* expression in blood is rare, occurring in only 5% of controls. Interestingly all eight unaffected *SMN1*-deleted individuals were female. Despite extensive research, the molecular cause for this gender-specific protective effect still remains unknown. We found that *PLS3* is highly expressed in spinal cord, associates with *SMN*, and that both proteins are part of a large multi-protein complex in spinal cord. Both *PLS3* and *SMN* are present at similar subcellular locations in primary motor neurons and increase in expression during neuronal differentiation. As an actin bundling protein, *PLS3* influences the F-actin level known to be important for axonal outgrowth and guidance. *PLS3* knock-down in neuronal differentiated PC12 cells severely affects axonal growth whereas *PLS3* over-expression induces axonal growth. Most importantly, over-expression of *PLS3* rescues the axonal growth defect caused by reduced *SMN* levels in neuronal differentiated PC12 cells, in primary motor neurons of SMA mouse embryos

and in an *in vivo* zebrafish SMA-model. Our data strongly support the view that the involvement of *SMN* in axonal outgrowth and pathfinding is the major pathogenic defect in SMA. The discovery of *PLS3* as a protector against SMA provides the opportunity to identify novel regulatory mechanisms that may act specifically in motor neurons, and which may be useful for understanding the molecular pathogenesis of other motor neuron diseases as well. The results may also help to identify novel targets for SMA therapy. Our discovery signifies a major breakthrough in medical genetics and represents the first report ever of a fully protective modifier for a Mendelian disorder in humans.

#### PL3.1

##### Systematic resequencing of the coding exons of the X chromosome in X-linked Mental Retardation

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Mental retardation (MR) affects 1-3% live births and has both genetic and non-genetic causes. A proportion of cases with genetic abnormalities are attributable to mutations of genes on the X chromosome. Although several X-linked MR (XLMR) genes have been reported, identification of more by conventional approaches is problematic because mutations of many genes cause MR and their associated phenotypes are similar. Here, we have implemented a new strategy in which the coding exons of X chromosome genes (~1Mb DNA per sample) have been systematically resequenced for disease-causing variants in individuals from more than 200 XLMR families. The strategy has yielded several new XLMR genes. However, many families remain to be explained. The study also indicates that loss of function of ~1% of X-genes is compatible with apparently normal existence. To our knowledge, this is the largest resequencing study to identify human disease genes thus far conducted. The results highlight issues that will be faced in the future by whole genome screens for rare disease-causing variants.

#### PL3.2

##### Cap-Analysis Gene Expression (CAGE) analysis of transcriptional complexity and regulation

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The genome sequence is an invaluable resource, yet it is still hard to identify all its encoded RNAs and regulatory regions. Despite there are about 20,000 protein coding genes, 63% to 93% of the mammalian genomes is expressed, mainly producing non-coding RNAs, starting from more than 230,000 core promoters. These are identified with CAGE (cap-analysis gene expression), resulting in a finely redefinition of the promoter structure.

The development of novel generation of sequencing instruments allows addressing the RNA complexity at a much higher definition. We are using multiple platforms (454 Life Science, Illumina/Solexa and ABI-SOLiD sequencers) to produce hundreds of millions of functional sequencing tags, with particular focus on CAGE and short RNA libraries. Sequencing of deep-CAGE libraries, which contain 1 to more than 10 millions tags, produces much detailed and sensitive data than microarrays at a progressively decreasing cost. We are broadening the CAGE applications to the ENCODE project and have also miniaturized the protocol, to work with RNA extracted from less than 1000 cells (neurons).

CAGE analysis allows correlating promoter elements to transcriptional control, as demonstrated by reconstructing the transcriptional network of differentiation of THP-1, a human myeloid leukemia cell in the Fantom 4 project in an integrated approach. We provide the first description of the dynamics and architecture of a human transcriptional network undergoing transition from a proliferating to a differentiated state

#### PL3.2

##### High-throughput SNP genotyping and ultra-high-throughput sequencing for genome-wide association studies

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In the course of the last 2 years the Centre National de Génotypage (CNG) has run numerous genome-wide association (GWA) studies us-

ing its high throughput genotyping platforms. We have widely applied different formats of Illumina Infinium and Affymetrix arrays. The CNG genotyping platforms are embedded in a system of highly standardized DNA handling and QC, LIMS, data QC, statistical analysis and OPERON, a bioinformatic knowledge database. Our studies have delivered many striking results, such as ORMDL3, a strong candidate gene associated with childhood asthma, PTGER4 a candidate gene associated with Crohn's disease, BCL11A a strong candidate gene associated with sickle cell disease and β-thalassemia, and others. The identification of associated genes was accelerated by a database of genome-wide association of global gene expression. Recently we managed to identify an intriguing group of genes that constitute sub-units of a nicotinic acetylcholine receptor on 15q25 associated with lung cancer susceptibility.

To gain better understanding of the regions of the genome that show association with a phenotype we are currently using a 2nd generation DNA sequencing platform based on Illumina Genome Analyzers. We have adapted efficient methods for the enrichment of regions of interest. The 2nd generation DNA sequencing methodology is well suited for the analysis of DNA samples enriched for particular genomic regions of interest. Due to the technical limitations of these technologies, their application for capturing structural variation such as short range length polymorphisms (microsatellites and minisatellites), duplications and inversion requires work arounds. We believe that a further paradigm shift to a 3rd generation of DNA sequencing technology will be required for cost-effective whole genome sequencing. This technology will have to be able to analyze clonal DNA molecules over long distances (5 - 30 kb). The European Community is funding an effort to develop such a technology through its FP7 programme READNA.

### PL4.1

#### Distinguished Speaker Lecture: Systems Biology and Systems Medicine

L. Hood;

President, Institute for Systems Biology, Seattle, WA, United States.

The grand challenge for biology and medicine in the 21st century is complexity. A currently emerging paradigm change is the idea that

biology is an informational science and that most biological information is mediated by dynamical biological networks. The systems approach to biology and medicine is a general category of approaches that appear to be very effective in dealing both with biological circuits and hence with biological complexity. Systems approaches require a truly cross-disciplinary environment and the effective integration of biology, technology and computation/mathematics. I will discuss my views of systems biology. Then I will discuss a systems approach to one disease, prion disease in mice, and demonstrate how it profoundly alters our views of disease—with regard to understanding disease pathophysiology as well as new approaches to diagnosis, therapy and eventually prevention. Then I will talk about the emerging measurement technologies that are the foundation of P4 medicine, as well as some of the pioneering computational and mathematical tools that will be necessary to usher in this revolution in medicine. The view of biology as an information science, the systems approach to disease, the new measurement and visualization technologies and the evolving mathematical/computation tools will catalyze this paradigm change in medicine. I will make five predictions: 1) our current largely reactive medicine will be transformed to a predictive, preventive, personalized and participatory (P4) medicine over the next 10 to 20 years, 2) this will lead to the digitalization of medicine (extracting information from single cells, single molecules and single individuals) with even more profound implications for society than the digitalization of communications and information technologies, 3) systems medicine and its digitalization will dramatically turn around the slope of ever increasing healthcare costs to the point that the developed world will be able to export its P4 medicine to the developing world, 4) P4 medicine will necessitate fundamental changes in the business plans of virtually every sector of the healthcare industry and 5) this new world of medicine will be propelled forward by carefully chosen strategic partnerships—across all sectors of science—academia, industry, government laboratories, independent research institutes, etc—and that these partnerships will be international. ISB hopes to play an important role in catalyzing a series of these strategic partnerships.

## ESHG CONCURRENT SYMPOSIA

## S01.1

## Dissection of structural variation in common human disease

*X. Estivill;*

Genes and Disease Program, Center for Genomic Regulation (CRG), Barcelona National Genotyping Center (CeGen), Public Health and Epidemiology Network Biomedical Research Center (CIBERESP), Pompeu Fabra University (UPF), Barcelona, Spain.

CNVs represent a new common source of genetic variability in individuals (recognized by Science as the breakthrough of 2007), which might constitute susceptibility factors for the onset, progress and severity of complex diseases. CNVs could directly affect the dose of certain genes or modify loci that regulate the expression of relevant genes, therefore providing important clues for disease and phenotype variability. We are using and implementing multiple technologies to further analyze CNVs potentially involved in several complex disorders, mainly psychiatric diseases, neurodegenerative diseases and inflammatory disorders. Functional validation of CNVs with respect to disease needs: a/ verification that the genomic variants are associated to changes in the expression of a gene product at the mRNA and protein levels; and b/ characterization of the physiological consequences associated to changes in the dose of a gene, which might contribute to specific traits of the disease. We have identified several genomic regions that contain CNVs that are common in the population and that could have an enormous impact in disease predisposition. We have preliminary results on the identification of CNVs for several neurological, neuropsychiatric and inflammatory disorders, and we are characterizing such genomic regions and performing genome scans to uncover the variability landscape of these disorders.

## S01.2

## Gene copy number variation and common human disease

*T. J. Aitman;*

Physiological Genomics and Medicine Group, MRC Clinical Sciences Centre and Imperial College, London, United Kingdom.

Gene copy number variation is now well recognised as a source of sequence variation in the genome of humans and other mammals. During positional cloning studies to identify genes for insulin resistance and autoimmune glomerulonephritis in the rat, we showed that gene copy number variants, at the *Cd36* and *Fcgr3* gene loci respectively, contributed to disease susceptibility in the rat model. In humans, we went on to show that low copy number of *FCGR3B*, an orthologue of rat *Fcgr3*, was associated with glomerulonephritis in the autoimmune disease systemic lupus erythematosus (SLE). More recently we found that low *FCGR3B* copy number predisposes to development of SLE itself and to development of the systemic autoimmune diseases microscopic polyangiitis and Wegener's granulomatosis. These studies provide direct evidence for the importance of heritable variation in gene copy number in the evolution of genetically complex phenotypes, including susceptibility to a range of common human diseases.

## S01.3

## Beta-defensin copy number variation: measurement, diversification and association with psoriasis

*J. A. L. Armour;*

Institute of Genetics, Nottingham, United Kingdom.

In the current excitement surrounding the recent discoveries from case-control association studies, it is essential to avoid overenthusiastic interpretation of error-prone data. Although this is still an important consideration for SNP typing, it is of particular concern in assessing the role of copy number variation, for which the development of typing technology satisfactory for case-control association studies is still in its early stages. I will address the importance of accuracy (as well as throughput) in measuring copy number, with reference to our own PRT methods applied to beta-defensin variation on 8p23.1. This copy number variation involves a cluster of seven defensin genes, presumed to act as antimicrobials, but which may have a wider spectrum of functions; copy number variation is commonly over the range between 2 and 7 copies per diploid genome. The accuracy of the typing methodology has been essential in discovering an association between beta-defensin copy number and psoriasis, as well as in revealing an unexpected and highly unusual mechanism for generating variation in the copy number of these genes.

## S02.1

## Mutation specific therapy: The CF experience

*E. Kerem;*

*Hadassah University Hospital, Jerusalem, Israel.*

CFTR mutations cause defects of CFTR protein production and function by different molecular mechanisms. The mutations can be classified according to the mechanisms by which mutations disrupt CFTR function. This understanding of the different molecular mechanism of CFTR dysfunction provides the scientific basis for development of targeted drugs for mutation specific therapy of CF. Class I mutations are nonsense mutations that result in the presence of premature stop codon that leads to the production of unstable mRNA or the production of a short truncated protein that is not functional. Drugs such as the aminoglycoside antibiotics and PTC124 can suppress premature termination codons by disrupting translational fidelity and allowing the incorporation of an amino acid, thus permitting translation to continue to the normal termination of the transcript. Class II mutations cause impairment of CFTR processing and folding in the Golgi. As a result the mutant CFTR is retained in the ER and eventually targeted for degradation by the quality control mechanisms. Chemical and molecular chaperones can stabilize protein structure, and allow it to escape from degradation in the ER and be transported to the cell membrane. Class III mutations disrupt the function of the regulatory domain. CFTR is resistant to phosphorylation or ATP binding. CFTR activators can overcome the affected ATP binding through direct binding to a nucleotide binding fold. In patients carrying class IV mutations, phosphorylation of CFTR results in reduced chloride transport. Increases in the overall cell surface content of these mutants might overcome the relative reduction in conductance. Activators of CFTR at the plasma membrane may function by promoting CFTR phosphorylation, by blocking CFTR dephosphorylation, by interacting directly with CFTR, and/or by modulation of CFTR protein-protein interactions. Class V mutations affect the splicing machinery and generate both aberrantly and correctly spliced transcripts, the level of which vary among different patients and among different organs of the same patient. Splicing factors that promote exon inclusion or factors that promote exon skipping can promote increase of correctly spliced transcripts, depending on the molecular defect. Inconsistent results were reported regarding the required level of corrected or mutated CFTR that has to be reached in order to achieve normal function.

## S02.2

## Synthetic lethal approaches to the development of new therapies for cancer

*A. Ashworth;*

*Breakthrough Breast Cancer Research Centre, The Institute of Cancer Research, London, United Kingdom.*

About one in nine women in the Western world develop cancer of the breast and at least 5% of these cases are thought to result from a hereditary predisposition to the disease. Two breast cancer susceptibility (*BRCA*) genes have been identified and mutations in these genes account for most families with four or more cases of breast cancer diagnosed before the age of 60. Women who inherit loss-of-function mutations in either of these genes have an up to 85% risk of breast cancer by age 70. As well as breast cancer, carriers of mutations in *BRCA1* and *BRCA2* are at elevated risk of cancer of the ovary, prostate and pancreas. The genes are thought to be tumour suppressor genes as the wild-type allele of the gene is observed to be lost in tumours of heterozygous carriers. Both *BRCA1* and *BRCA2* have significant roles in the maintenance of genome integrity via roles in the repair of DNA damage via homologous recombination. The specific DNA repair defect in *BRCA*-mutant cells provides opportunities for novel therapeutic approaches based on selective inhibition of functionally interacting repair pathways, in particular by inhibition of the enzyme PARP. Here I will describe recent work defining determinants of sensitivity and resistance to PARP inhibitors, as well as the application of the synthetic lethal approach to other cancer types.

### S02.3

#### Small-molecule therapy for Cystic Fibrosis

A. S. Verkman;

Department of Medicine and Physiology, University of California, San Francisco, San Francisco, CA, United States.

The Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a cAMP-activated chloride channel expressed in epithelia in the lung, intestine, pancreas, testis and other tissues, where it facilitates transepithelial fluid transport. In the intestine CFTR provides the major route for chloride secretion in certain diarrheas. Mutations in CFTR cause the hereditary disease cystic fibrosis, where chronic lung infection and deterioration in lung function cause early death. CFTR is a well-validated target for development of inhibitors for therapy of secretory diarrheas and polycystic kidney disease, and activators for therapy in cystic fibrosis. Our lab has identified and optimized small molecule inhibitors of CFTR, as well as activators of deltaF508-CFTR, the most common mutant CFTR causing cystic fibrosis. High-throughput screening of small molecule collections utilizing a cell-based fluorescence assay of halide transport yielded thiazolidinone and glycine hydrazide CFTR inhibitors that block enterotoxin-mediated secretory diarrhea in rodent models, including a class of non-absorbable inhibitors that target the CFTR pore at its external entrance. Nanomolar-potency benzothiophene, phenylglycine and sulfonamide potentiators were identified that correct the defective gating of deltaF508-CFTR chloride channels, restoring their function to that of wildtype CFTR. Several classes of correctors of defective deltaF508-CFTR cellular misprocessing were discovered, including bisaminomethylbithiazoles, that improve mutant CFTR folding and facilitate its stability and targeting to the cell plasma membrane, restoring cAMP-stimulated chloride permeability. Small-molecule modulators of CFTR function are in development for the treatment of cystic fibrosis, secretory diarrhea and polycystic kidney disease.

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### S03.1

#### Is the era of genetic counseling over?

S. Kessler;

Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, CA.

### S03.2

#### Medical genetic services in developing nations

A. Christianson;

Division of Human Genetics, National Health Laboratory Services & University of the Witwatersrand, Johannesburg, South Africa.

An expert advisory group of the WHO recognized in the early 1980's that health (epidemiological) transition would require developing countries, within the foreseeable future to develop medical genetic services. To that end the expert advisory group developed an approach cogent for developing nations that focused on community based medical genetic services integrated into primary health care and closely linked to secondary and tertiary health care services.

In the latter half of the last century these approaches were refined, to an extent based on services that were being established in a few developing countries. This decade several middle-income nations have recognized the need to develop medical genetic services, initially for the care and prevention of birth defects. Following the approach proposed by the WHO these nations are developing medical genetic services, some with assistance from the March of Dimes and the World Alliance of Organizations for Prevention and Treatment of Genetic and Congenital Conditions (WAO). Numerous barriers to the establishment of these services still exist, but are being overcome.

### S03.3

#### Personalized Medicine and Genetic Services: The US Model

M. Aspinall;

Genzyme Corporation, Westborough, MA, United States.

A revolution is underway in the life sciences and health care industry. Fueled by the mapping of the human genome and a deepening understanding of human genetic variation and ongoing advances in diagnostics are expanding our understanding of the molecular basis of disease. Health care delivery is beginning to shift from trial-and-error medicine to patient-centric medicine. Patient care is becoming focused on highly targeted and individualized diagnostic and treatment regimens. The use of genetic services is expanding in all areas. This revolution is known as personalized medicine.

Personalized medicine is a movement away from defining diseases by their symptoms and locations within the body, and toward understanding them through their underlying genetic causes. With a successful transition to this model, more specific disease diagnoses will occur, resulting in a personalized treatment plan for individual patients. Costs will be reduced through more accurate diagnosis, improved drug efficacy with fewer adverse drug reactions as well as improved patient drug compliance. This trend to personalized medicine and increased use of genetic services is occurring today in the field of oncology and will expand to all areas of medicine.

The field of genetics services - both testing and counseling - is essential to the implementation of personalized medicine. Without these services, physicians and other healthcare providers will not be able to access the information necessary to make the most informed choices for patients.

Personalized medicine, however, must overcome multiple challenges if it is to fully adopted. There are significant challenges involving physician and patient education and acceptance. An expanded understanding of genetics and diagnostics and its impact on patient care will be necessary for all healthcare providers. A health information infrastructure must be able to accommodate the accumulation and analysis of expanding patient and pharmacogenomic data.

In this talk, I will review the current status of personalized medicine in the United States including examples of its current impact on patient care. I will also describe the current United States diagnostic market including the current distribution systems and infrastructure design.

### S03.4

#### The Clinical Use of Genetic and Molecular Biomarkers: A Public Health Perspective

R. Zimmern;

PHG Foundation Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom.

Advances in genomic science have led to a much greater knowledge of disease mechanisms and the development of novel technologies such as genetic tests and molecular biomarkers. Unlike the well trodden pathway that exists for pharmaceutical products, we do not have within Europe (or anywhere across the developed world) an effective framework for their clinical evaluation. The absence of mechanisms for generating the necessary data, or of institutions that focus on their analysis, and the lack of policy about the respective responsibilities or the public and commercial sectors for their establishment and funding are major concerns for the practice of medicine and for public health. The lecture will argue that an approach to the introduction of tests into clinical practice based on clinical judgement is longer sustainable. The complexities of modern diagnostics will require a more formal and innovative approach. Tests will require explicit evaluation to identify the clinically valid and useful. Biomarkers that are predictive of complex disease before its development, in contrast to diagnostic or prognostic markers or to genetic tests for high penetrance single gene disorders, pose a particular challenge. The low relative risks that these show, and the fact that each individual biomarker will be neither necessary nor sufficient for the development of disease suggests that a different approach to their assessment will be needed.

#### S04.1 microRNA Regulation of Cardiac Development and Disease

**D. Srivastava:**

Gladstone Institute of Cardiovascular Disease, UCSF – Dep. of Pediatrics and Biochemistry & Biophysics, San Francisco, CA, United States.

Gradients of signaling and transcription factors result in distinct cellular responses during organ formation suggesting that the precise dose of major regulatory proteins must be tightly controlled. MicroRNAs (miRNAs) are phylogenetically conserved small RNAs that regulate translation or stability of target messenger RNAs providing a mechanism for protein dose regulation. Studies in our lab of multiple cardiac-enriched miRNAs reveal that they coordinate decisions of cellular proliferation, differentiation and response to stress via intricate transcriptional and translational networks. In addition to our previous work demonstrating the role of miR-1 in differentiation of mouse and fly cardiac progenitors, we found that targeted deletion of miR-1-2 in mouse causes defects in cardiac morphogenesis as well as cardiac conduction and cell cycle abnormalities. Consistent with this finding, manipulation of miR-1 and the co-transcribed miR-133 in mouse and human embryonic stem cells revealed that these miRNAs can be used to guide pluripotent stem cells into mesodermal cells and ultimately into the cardiac lineage, while repressing neuroectodermal and endodermal differentiation. Finally, novel approaches of miRNA target identification to explain the mechanisms underlying the described effects of cardiac miRNAs will be discussed.

#### S04.2

#### A rapidly evolved RNA gene may have played a role in the evolution of the cerebral cortex

**D. Haussler:**

Center for Biomolecular Science & Engineering, University of California, Santa Cruz, CA, United States.

We have scanned the human genome for segments that have been under negative selection during most of mammalian evolution, but experienced a burst of changes during the last few million years of human evolution. The most dramatic such segment occurs in a previously unstudied RNA gene expressed specifically in the Cajal-Retzius neurons in the developing cerebral cortex, during the time these neurons guide the development of the 6-layer cortical structure. Examples like this demonstrate the power of computational reconstruction of the evolution of the human genome, and argue that changes in non-coding functional regions may have played a significant role in the molecular events that forged our species.

#### S04.3

#### The RNAi strategy in Cancer: Towards the Achilles Heel of Cancer

**R. L. Beijersbergen:**

The Netherlands Cancer Institute , Division of Molecular Carcinogenesis and NKI Robotics and Screening Center, Amsterdam, Netherlands.

The development of the RNA interference (RNAi) technology has changed the way how we approach target discovery and validation in cancer research. The potential to study the consequence of the inactivation of each individual gene is a very effective tool to identify novel targets. In addition, high content imaging allows us to identify novel components of cellular pathways involved in complex cellular phenotypes in a high throughput manner. The combination of RNA interference and high content imaging will lead to the discovery of a new class of targets that can be used for development of novel cancer therapies or to improve existing therapies.

We have constructed a large set of retroviral vectors encoding more than 50.000 shRNAs, which target 15.000 different human or mouse genes for suppression. This RNA interference library has been used to identify genes involved in major cellular pathways such as the p53 tumor suppressor pathway. In particular we have focused on genes that modulate the cytotoxic response to small molecules that target the MDM2-p53 interaction. In addition we have developed novel screening methods with the use of shRNA libraries and DNA micro-arrays to be able to rapidly screen large numbers of shRNA vectors. This technology is applied to identify the mechanism of action of novel anti-cancer drugs and to identify genes involved in resistance to anti-cancer drugs.

Recently, we have extended our efforts into synthetic siRNA screens to

allow genome wide single well high throughput screening with the goal to study more complex phenotypes and, importantly, to identify targets that upon inhibition would only affect tumor cells where normal cells would remain unaffected. The concept that a particular mutation has deleterious consequences under specific conditions is known as synthetic lethality. Two genes are defined as synthetic lethal when cells die if they have both genes mutated but can survive if either gene alone is mutated. The approach of exploring synthetic lethal gene-gene interactions is attractive because it turns a hallmark of cancer cells, specific mutations, into a weakness that can be explored therapeutically. We explore the existence of synthetic lethal interactions with tumor specific genetic alterations and large scale siRNA screens.

These approaches illustrate the power of RNAi to gain insight in the mode of action of novel cancer drugs with the goal to accelerate their development and as a powerful way to identify a whole new class of more specific and more efficient anticancer drugs.

#### S05.1

#### Guidelines for the clinical management of Lynch syndrome and adenomatous polyposis

**H. F. A. Vasen:**

Department of Gastroenterology & Hepatology, Leiden University Medical Centre, Leiden, Netherlands.

The Lynch syndrome (LS)(HNPCC) is characterized by the development of colorectal cancer (CRC), endometrial cancer and various other cancers and is caused by a mutation in one of the mismatch repair (MMR) genes: *MLH1*, *MSH2*, *MSH6* or *PMS2*. Familial adenomatous polyposis (FAP) is a well-described inherited syndrome, characterized by the development of hundreds to thousands of adenomas in the colorectum. The syndrome is caused by mutations in the *APC*-gene or the *MUTYH*-gene. Both syndromes (LS, FAP) are responsible for at least 5-7 % of all cases of CRC. Since 2006, annual workshops were organized by a group of European experts in hereditary gastrointestinal cancer (the Mallorca group) aiming to establish guidelines for the clinical management of hereditary CRC syndromes. Thirty-one experts from nine European countries participated in these workshop. Prior to the meeting, various participants prepared the key management issues of debate according to the latest publications. A systematic literature search using Pubmed and the Cochrane Database of Systematic Reviews, reference lists of retrieved articles, and manual searches of relevant articles was performed. During the workshop all recommendations were discussed in detail. Part of the guidelines will be discussed. Moreover, the results of recent studies on cancer risk and experience of longterm surveillance for CRC in the Lynch syndrome will be presented.

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2. H.F.A. Vasen & G. Mösllein & the Mallorca group. Guidelines for the clinical management of Familial adenomatous polyposis. Gut 2008; 57:704-13

#### S05.2

#### Evaluation of breast and ovarian cancer screening programmes in BRCA1 and BRCA2 mutation carriers: the UK, Norwegian and Dutch experience

**D. G. Evans<sup>1</sup>, K. N. Gaarenstroom<sup>2</sup>, D. Stirling<sup>3</sup>, A. Shenton<sup>1</sup>, L. Maehle<sup>4</sup>, A. Dørum<sup>4</sup>, M. Steel<sup>5</sup>, F. Laloo<sup>1</sup>, J. Apold<sup>6</sup>, M. E. Porteous<sup>3</sup>, H. F. A. Vasen<sup>7</sup>, C. J. van Asperen<sup>8</sup>, P. Moller<sup>9</sup>;**

<sup>1</sup>Medical Genetics Research Group and Regional Genetics Service, University of Manchester and Central Manchester and Manchester Children's University Hospitals NHS Trust, St Mary's Hospital, Manchester, United Kingdom, <sup>2</sup>Department of Gynaecology, Leiden University Medical Center, Leiden, Netherlands, <sup>3</sup>South East of Scotland Genetics Service, Western General Hospital, Edinburgh, United Kingdom, <sup>4</sup>Section for Inherited Cancer, Department of Medical Genetics, Rikshospitalet Radiumhospitalet Clinical Center, Oslo, Norway, <sup>5</sup>University of St Andrews, Bute Medical Buildings, St Andrews, United Kingdom, <sup>6</sup>Centre of Medical Genetics and Molecular Medicine, Haukeland University Hospital, and Institute of Clinical Medicine, University of Bergen, Bergen, Norway, <sup>7</sup>The Netherlands Foundation for the Detection of Hereditary Tumours and the Department of Gastroenterology, Leiden University Medical Center, Leiden, Norway, <sup>8</sup>Center for Human and Clinical Genetics, Department of Clinical Genetics,

*Leiden University Medical Center, Leiden, Norway.*

The management of the high risk of breast and ovarian cancer in female BRCA1/BRCA2 mutation carriers remains a vexing issue. In order to provide alternatives to risk reducing surgery, surveillance programmes have to detect the disease sufficiently early to provide a very high likelihood of cure. In particular there have been substantial doubts about the ability of routine annual mammography and ovarian ultrasound plus serum CA125 to meet this goal. Collaborative research between the UK, the Netherlands and Norway has enabled us to provide a more accurate assessment of the likelihood of cure within these programmes. Additionally the benefits of MRI screening for breast cancer have been demonstrated by National studies in each country.

To assess the effectiveness of annual ovarian cancer screening (transvaginal ultrasound and serum CA125 estimation) in reducing mortality from ovarian cancer in women at increased genetic risk. A cohort of 3532 women at increased risk of ovarian cancer was screened at five centres between January 1991 and March 2007. Survival from diagnosis of ovarian cancer was calculated using Kaplan-Meier analysis and compared for proven *BRCA1/2* carriers with non-carriers and whether the cancer was detected at prevalence or post prevalent scan. Screening was performed by annual transvaginal ultrasound and serum CA125 measurement

64 epithelial ovarian malignancies (59 invasive and 5 borderline), developed in the cohort. 26 tumours were detected at prevalent round, there were 27 incident detected cancers and 11 interval. Sixty-five percent of cancers were stage 3 or 4, however, stage and survival were little different for prevalent versus post prevalent cancers. Five year and 10-year survival in 49 *BRCA1/2* mutation carriers was 58.6% (95% CI 50.9-66.3%) and 36% (95% CI 27-45%), which was significantly worse than for 15 non *BRCA*-carriers (91.8% (95% CI 84-99.6%) both 5 and 10-year survival p=0.015). Annual surveillance, by trans-vaginal ultrasound scanning and serum CA125 measurement in women at increased familial risk of ovarian cancer is ineffective in detecting tumours at a sufficiently early stage to substantially influence survival in *BRCA1/2* carriers.

A collaborative study between Norway and the UK has shown that survival in prospectively detected breast cancers in *BRCA1* carriers in an annual mammography screening programme is significantly worse than for *BRCA2* and other familial groups. It appears this may be due to relatively poor survival for small node negative cancers. Some of this poor survival may be attributable to the failure to treat these small tumours with chemotherapy. This is particularly relevant when assessing the benefits of MRI which in combination with mammography has >90% sensitivity at detecting breast cancers in an annual programme. The benefits of the combined approach for *BRCA2* appear robust with survival likely to exceed 90% for breast cancers identified in surveillance programmes.

### S05.3

#### Managing genetic risk: some issues for *BRCA1* and *BRCA2* mutation carriers

*N. Hallowell;*

*Public Health Sciences, University of Edinburgh, Edinburgh, United Kingdom.*

Men and women who have a number of relatives with breast, ovarian and prostate cancer in close relatives may be at risk of developing these diseases because they carry a dominantly inherited (*BRCA1/2*) mutation. These individuals need to reach a decision about managing their cancer risks. There are a number of risk management strategies available - which can be divided into familial and individual strategies. The former entails DNA screening - determining whether individuals and, subsequently, their relatives carry a mutation. The latter include: bodily surveillance (breast, ovarian and prostate screening), chemoprevention (e.g. tamoxifen, oral contraception), risk-reducing pre-symptomatic surgery (e.g. mastectomy, oophorectomy), which are available to at-risk confirmed and unconfirmed mutation carriers.

This presentation will present an overview of some of the main issues emerging from research which investigates the psychosocial implications of cancer risk management. I will argue that both risk management decisions and the process of managing risk have both an individual and familial dimension. Some of the factors influencing risk management decisions such as: gender, cancer status and stage in the lifecycle will be discussed as will the repercussions for identity of adopting particular risk management practices. Finally, some of the

implications of this research for service delivery in cancer genetics will be considered.

### S06.1

#### Common, low-penetrance breast cancer susceptibility alleles: the clinical relevance

*P. Pharoah;*

*University of Cambridge Dept of Oncology, Strangeways Research Laboratory, Cambridge, United Kingdom.*

Empirical, genome-wide association studies have discovered seven breast cancer susceptibility alleles that are common in the population. These findings have brought the promise of a "polygenic" approach to the prevention of breast cancer a step nearer. The risks conferred by individual loci are small, but risk alleles seem to act multiplicatively. As a result there is an approximately six-fold difference in risk of breast cancer between women carrying 14 risk alleles and those carrying no risk alleles at these loci. Overall, the distribution of relative risk in the population based on combinations of genotypes at these loci is approximately log-normal. The efficiency of population-based preventive programs, such as screening mammography, could be improved by targeting women at greatest risk based on genotype.

### S06.2

#### What GWAS have taught us about the genetic architecture and pathogenesis of the inflammatory bowel diseases (IBD).

*J. Rioux;*

*Université de Montréal and the Montreal Heart Institute Research Center, Montréal, QC, Canada.*

Crohn's disease and ulcerative colitis are debilitating, inflammatory diseases of the gastrointestinal tract, collectively known as the inflammatory bowel diseases (IBDs). Genetic studies have been particularly successful in the identification of genes for Crohn's disease. In fact, in 2001 one of the first successful discoveries of a causal gene for a complex trait was the identification of three genetic variants in the NOD2 gene that are associated with Crohn's disease as well as the identification of an associated haplotype on chromosome 5q31, known as IBD5, containing five genes: IRF1, SLC22A5, SLC22A4, PDLIM4, and P4HA2.

Recent GWAS of CD has identified and confirmed an additional set of nine genetic risk factors. These studies have since led to an international collaboration to combine the data from over 3,000 patients with CD, examined in the individual screens, in order to identify the most significant associations for replication in an independent set of nearly 4,000 patients with CD. This collaborative work has identified a minimum of 20 additional risk factors for susceptibility to CD. It is estimated that the 31 loci identified to date explain about 10% of the overall variance in disease risk; providing some indications of the extent of the complexity in genetic architecture for this chronic immune-mediated disease. Some of the pathogenic pathways newly identified by these GWAS include autophagy, novel innate immunity mechanisms, and T<sub>H</sub>17 mediated immune responses. Interestingly, a subset of the genes identified to date are also seen to influence other immune mediated diseases and thus provide a window into the disease-specific and shared pathogenic pathways. In this presentation we will discuss the results from these recent discoveries and some of conclusions that can currently be drawn from this data in terms of the genetic architecture and pathogenesis of IBD as a model for other complex human traits.

### S06.3

#### Title to be announced

*G. Abecasis;*

*University of Michigan, School of Public Health, M4132 SPH II, Ann Arbor, MI.*

### S07.1

#### Genomic encoding of positional identity

*H. Y. Chang;*

*Program in Epithelial Biology, Stanford University School of Medicine, Stanford, CA, United States.*

The problem of how genetic information gives rise to the spatial organization has long intrigued biologists. While cellular differentiation addresses the control of expression of specific genes within a cell, pattern formation addresses the spatial arrangement of distinct cell types. A major mechanism of pattern formation in the embryo is the

use of positional information. By linking differentiation programs to cell positions on a coordinate system, an assembly of cells can be programmed to develop into well-defined spatial patterns that are not easily perturbed by the removal or addition of cells. In contrast to embryonic development, the higher-order patterns of cellular specialization in adult animals and mechanisms of their maintenance are less well understood. Here I discuss progress in using genomic expression programs to understand the organization and mechanisms of pattern formation and maintenance in mammalian epithelia.

The major themes are the encoding positional identity in one class of cells and the transfer of this information by epithelial-mesenchymal interaction; the role of chromatin modifications in the fidelity of transcriptional memory, and the discovery of a class of long non-coding RNAs that regulate chromosomal domains of chromatin modification to enable position-specific gene expression.

### S07.2

#### Rapid high-resolution identification of balanced genomic rearrangements by 4C technology

M. Simonis<sup>1</sup>, P. Klous<sup>1</sup>, R. Galjaard<sup>2</sup>, E. Rijkers<sup>3</sup>, F. Grosveld<sup>4</sup>, J. Meijerink<sup>4</sup>, W. deLaat<sup>1</sup>;

<sup>1</sup>Department of Cell Biology, Erasmus MC, Rotterdam, Netherlands, <sup>2</sup>Department of Clinical Genetics, Erasmus MC, Rotterdam, Netherlands, <sup>3</sup>Department of Biochemistry, Erasmus MC, Rotterdam, Netherlands, <sup>4</sup>Department of Pediatric Oncology, Erasmus MC, Rotterdam, Netherlands.

The architecture of DNA in the cell nucleus is an emerging key contributor to genome function. To better understand how DNA is folded inside the cell nucleus, we recently developed 4C technology. 4C technology is a high-throughput technique that combines 3C (chromosome conformation capture) technology with tailored micro-arrays to uniquely allow for an unbiased genome-wide search for DNA loci that interact in the nuclear space (Simonis et al., *Nature Genetics* 2006). It is based on formaldehyde cross-linking and capturing of *in vivo* interacting DNA elements, which are subsequently ligated to each other and PCR amplified.

Here, we will show that 4C technology is also a powerful technique for the accurate identification of balanced and unbalanced genomic rearrangements. Balanced chromosomal rearrangements (inversions, translocations) frequently occur in the human population and can cause disease, but techniques for their rapid and accurate identification are missing. 4C technology accurately reconstructs at least 5-10 megabases of the one-dimensional chromosome sequence map around the selected genomic viewpoint. Changes in this physical map as a result of genomic rearrangements are therefore identified by 4C technology. We demonstrate that 4C detects balanced inversions and translocations, but also unbalanced rearrangements like deletions, at a resolution (~7kb) that allows immediate sequencing of the breakpoints. Breakpoints are identified even when they are 4 megabases away from the genomic viewpoint. We have applied 4C to samples from patients with congenital malformations and with T cell acute lymphoblastic leukemia (T-ALL). Using 4C, we have identified novel rearrangements underlying T-ALL. We will show that balanced rearrangements are identified also if they occur in a small subpopulation of cells. 4C technology therefore offers a novel high-resolution genomic approach that can efficiently identify balanced genomic rearrangements.

### S07.3

#### Integrative genomic approaches for the identification of regulatory variation underlying disease risk

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### S08.1

#### Deciphering Developmental Disorders

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Genetically determined disorders of development result in malformations (e.g. congenital heart defects), a dysmorphic appearance (i.e. unusual craniofacial appearance) and/or neurodevelopmental disability. They have a profound effect on the life and health of the individual and of their family. Many developmental disorders are caused by gene

mutations or larger chromosome rearrangements affecting gene copy number or regulation. However, in spite of expert clinical assessment and conventional chromosome analysis, most children with developmental disorders remain undiagnosed and indeed are undiagnosable using this methodology.

Recently it has become possible to screen patients for submicroscopic chromosome imbalance (microdeletions and microduplications) and to identify mutations in genes on a genome-wide scale. The application of comparative genomic hybridisation to DNA microarrays (array-CGH) has revolutionised our ability to identify small chromosome imbalances down to a few Kb not only in patients but also as normal copy number variation in unaffected individuals. A number of clinical laboratory centres worldwide are now applying genomic microarray technology to investigate a small proportion of patients with developmental delay, learning disability and congenital malformation. However the sporadic nature and rarity of the majority of these cases limits the ability of the individual clinician, working in isolation, to interpret the molecular findings from genome-wide array analysis. There is a great need for international collaboration to report and catalogue genotype-phenotype correlations such that clusters of individuals sharing similar genomic rearrangements and phenotypes can be identified. To facilitate such international collaboration, we have developed the DECIPHER database with the general aim of providing a clinical and research tool to:

- a. Aid in the interpretation of data from genomic microarray analysis e.g. the differentiation between pathogenic and polymorphic copy number changes
- b. Utilise the human genome map via the Ensembl genome browser to define genes involved in a specific microdeletion, microduplication, translocation or inversion
- c. Facilitate collaboration between clinical geneticists and molecular cytogeneticists using the world-wide-web to accelerate progress in the delineation of new syndromes and of gene function

### S08.2

#### Processes of allelic and ectopic recombination in the human genome

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Single molecule typing of sperm DNA allows very high resolution analysis of meiotic recombination events in human DNA and has revealed narrow crossover hotspots dominating the allelic recombination landscape, at locations that in general correlate well with regions of breakdown of linkage disequilibrium. All 36 hotspots characterised to date by sperm typing show a very similar morphology but vary hugely in recombination activity. Polymorphism between men in activity at specific hotspots is common, consistent with rapid evolutionary turnover of hotspots, but curiously appears to be restricted to less active hotspots, at least half of which show either quantitative variation or complete on/off polymorphism. These polymorphisms help identify local DNA sequence determinants and other factors that appear to influence hotspot activity. Single DNA molecule methods have also been developed to explore ectopic recombination between locally repeated DNA sequences. Analysis of the  $\alpha$ -globin gene cluster has revealed recombinational exchanges between the duplicated  $\alpha$ -globin genes leading to apparently reciprocal duplications and deletions, though with some evidence for an additional minor pathway of intramolecular deletion. These exchanges are surprisingly common, contrasting sharply with the rarity of rearranged chromosomes in most human populations and implying significant selection pressure against these rearrangements, despite the lack of obvious phenotypic effect in individuals carrying these copy number variants. Unlike recombination at allelic crossover hotspots, exchanges between  $\alpha$ -globin genes are not restricted to meiosis but also arise mitotically to give various classes of rearrangement whose frequencies can be erratically inflated by mutational mosaicism, even in the germline. Similar analysis of Lepore-type deletions in the  $\beta$ -globin gene cluster has provided further support for distinct allelic and ectopic recombination pathways, with evidence that a very active allelic recombination hotspot within the cluster is not responsible for driving ectopic exchanges.

**S08.3****Chromosome rearrangements and fusion genes in breast and other epithelial cancers****P. Edwards;***Department of Pathology, University of Cambridge, Hutchison-MRC Research Centre, Cambridge, United Kingdom.*

Chromosome translocations that form fusion transcripts or activate genes by promoter insertion are central to leukaemias, lymphomas, and sarcomas, but for various reasons have been neglected in the common epithelial cancers. It is now clear that at least some of the abundant chromosome rearrangements in the common cancers create fusion genes (reviewed by Mitelman et al, *Nature Reviews Cancer* 2007;7:233). Tomlins et al. (*Science* 2005;310:644) have shown that most prostate cancers have fusions of ETS transcription factors and Soda et al (*Nature* 2007;448:561) reported an EML4-ALK fusion present in around 7% of NSCLC lung cancers. We have undertaken a comprehensive analysis of chromosome rearrangements in breast cancer cell lines, mapping all rearrangements to 1Mb resolution or better (Howarth et al, *Oncogene* 2008, PMID18084325). We used 'array painting', in which chromosomes are isolated using a cell sorter (flow cytometer) and then hybridised to DNA microarrays to determine what parts of the genome are present in each chromosome. We found that many more translocations were balanced than expected: a total of nine reciprocal translocations in three cell lines completely analysed, with several other translocations balanced for at least one of the participating chromosomes. Many of the mapped breakpoints were in the kind of genes one would expect oncogenic translocations to target, and to date three in-frame fusion transcripts have been verified. It may be that gene fusions caused by chromosome rearrangement will prove to be as significant in common epithelial cancers as in leukaemias and sarcomas.

**S09.1****Intracellular Trafficking and Neurodegeneration - The SCA1/ataxin-1 Story****H. T. Orr;***Institute of Human Genetics, The University of Minnesota, Minneapolis, MN, United States.*

Spinocerebellar Ataxia type 1 (SCA1) is one of nine inherited disorders caused by a polyglutamine expansion in the affected protein. In SCA1 this expansion is in the ataxin-1 (ATXN1) protein. Nuclear localization of ATXN1 is implicated in the pathology of SCA1. Previous work showed that toxicity of mutant ATXN1 is due to soluble protein and its interacting proteins. Thus, polyglutamine-expanded mutant ATXN1 is able to interact with several other nuclear proteins and incorporate into native complexes similar to wild type protein. We identified partners of ATXN1 that interact with it in a manner dependent on two criteria necessary for toxicity: polyglutamine expansion and phosphorylation at serine 776. Polyglutamine expansion as well as phosphorylation of serine 776 in ATXN1 favors the formation of a particular protein complex containing a putative regulator of RNA splicing RBM17. We further found that changing the serine at position 776 to an aspartic acid (a substitution that can mimic phosphorylation) renders a wild type allele of ataxin-1 with 30 glutamines pathogenic in vivo. Mice expressing ataxin-1 30Q-D776 have a phenotype very similar to that seen in mice expressing ataxin-1 82Q-S776. These findings demonstrate the glutamine expansion in ATXN1 enhance protein/protein interactions that are normally regulated by its phosphorylation at serine at position 776 and that polyglutamine-induced misregulation of S776 phosphorylation and subsequent alterations in nuclear trafficking underlie SCA1 pathogenesis.

**S09.2****Polyneuropathies and axonal trafficking****K. A. Nave;***Department of Neurogenetics, Max Planck Institute of Experimental Medicine, Goettingen, GERMANY.***S09.3****'Neurotic Yeast' and the Molecular Basis of Parkinson's Disease****T. F. Outeiro;***Instituto de Medicina Molecular, Cell and Molecular Neuroscience Unit, Lisboa, Portugal.*

Aging is the major known risk factor for Alzheimer's disease (AD) and Parkinson's disease (PD), but genetic defects have been associated with familial cases. Huntington's disease (HD) is a purely genetic neurodegenerative disorder, where mutations in the *IT15* gene, encoding for the protein huntingtin, determine the development of the disease. A common hallmark to many neurodegenerative diseases is the presence of proteinaceous inclusions inside neuronal populations, which are selectively affected in each disorder. Lewy bodies, made of  $\alpha$ -synuclein in PD, and huntingtin inclusions, in HD, are but a few examples of protein aggregates deposited inside neurons. Whether inclusions are themselves toxic or actually cytoprotective is still under current debate, but it is widely accepted that protein misfolding and oligomerization are central molecular events in these diseases.

Molecular genetic approaches using different model organisms, from yeast to mammalian cell culture and mouse models, coupled with advanced microscopy techniques resulted in a detailed characterization of the pathways and events involved in cytotoxicity.

Using the budding yeast *Saccharomyces cerevisiae* as a 'living test tube' we were able to unveil fundamental aspects of  $\alpha$ -synuclein biology. Powerful yeast genetic screens enabled us to identify several molecular pathways as playing central roles in the toxicity induced by  $\alpha$ -synuclein. Genes involved in intracellular trafficking, lipid metabolism, and oxidative stress, were among the most highly represented categories. With this knowledge at hand, we are applying a variety of tools to unravel the molecular basis of neurological disorders associated with protein misfolding, with the goal of developing novel avenues for therapeutic intervention.

**S10.1****In utero stem cell transplantation: where are we now?****T. H. Bui;***The Karolinska Institute, Department of Molecular Medicine, Clinical Genetics Unit, Karolinska University Hospital, Stockholm, Sweden.*

In the last 35 years, extensive progress has been made in the prenatal diagnosis of genetic disorders. In contrast, success in fetal therapeutic interventions has been more limited.

One of the basic tenets of immunology is *learned self tolerance*: the ability, at the cellular and molecular levels, to recognise "self" and to eliminate that which is "foreign" must be "learned" during fetal life. This paradigm has served to support the concept of *in utero* transplantation (IUT), a promising approach with the potential to effectively treat fetuses with a variety of genetic defects. The rationale is to take advantage of normal events during haematopoietic and immunological ontogeny to facilitate allogeneic stem cell engraftment at an early stage of pregnancy, before permanent damage has occurred to the fetus. Clinical success has been realised, so far, in only fetuses with severe combined immunodeficiency syndromes. More recently, research has focused on mesenchymal stem cells (MSCs). Like adult bone marrow-derived MSCs, fetal liver-derived MSCs appear to be non-immunogenic both *in vitro* and *in vivo*. Both adult and fetal MSCs retain multilineage potential to form e.g. cartilage, bone, adipose and muscular tissues on induction. The first cases of IUTs using fetal MSCs for fetuses with severe osteogenesis imperfecta have been performed in our Centre.

Lessons learned from animal studies and clinical cases have contributed in defining new strategies to possibly overcome barriers to engraftment or tolerance in the fetus. The experience gained in IUTs is likely to benefit also the new experimental field of intrauterine gene therapy. Clearly, ethical issues relating to these new frontiers of medicine need also to be addressed.

**S10.2****Non-invasive prenatal diagnosis of Down syndrome: a challenging puzzle in circulating fetal nucleic acid research****R. Chiu:**

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In 1997, our group discovered the existence of cell-free fetal DNA and subsequently fetal RNA in the plasma of pregnant women. Such circulating fetal nucleic acids represent a convenient source of genetic material from the unborn child that could be sampled non-invasively and simply through the collection of a maternal blood sample. This approach is thus a safe alternative to conventional methods which rely on fetal cell sampling through invasive procedures such as amniocentesis. We showed that the detection of circulating fetal nucleic acids could potentially be applied to the non-invasive assessment of fetal blood group status, sex-linked genetic diseases and beta-thalassae-mia as well as the monitoring of pregnancy-related complications, such as preeclampsia. Among these applications, the non-invasive assessment of fetal rhesus D status has been adopted as a routine clinical test in a number of centres in Europe. However, Down syndrome is the main reason for couples opting for prenatal diagnosis. Due to the cell-free nature of fetal DNA/RNA in maternal plasma, development of non-invasive definitive diagnostic methods for Down syndrome had been a challenge. After tackling this puzzle for a decade, we have recently developed strategies to directly assess the dosage of chromosome 21 from maternal plasma allowing for direct non-invasive detection of fetal Down syndrome. One method, termed the RNA-SNP approach, is based on determining the ratio between polymorphic alleles of a placental expressed mRNA derived from chromosome 21 in maternal plasma. Another method, termed the relative chromosome dosage approach, is based on the detection of an excess of chromosome 21 DNA sequences with respect to a reference chromosome in maternal plasma. These approaches have brought us closer to realising the goal of non-invasive prenatal diagnosis of fetal Down syndrome.

**S10.3****The regulation of prenatal and preimplantation genetic diagnosis in the UK****E. Jackson:**

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This paper will explain how the Human Fertilisation and Embryology Authority (HFEA) regulates preimplantation genetic diagnosis (PGD) in the UK. Prenatal genetic diagnosis is not separately regulated in the UK, although abortion law provides a special ground for abortion on the grounds of abnormality, and this initially was used as a model for the regulation of PGD.

PGD is not specifically mentioned in the UK's legislation - the Human Fertilisation and Embryology Act 1990. Instead the HFEA has laid down the circumstances in which PGD may be used through its Code of Practice, currently in its 7<sup>th</sup> edition. In short, there has to be a significant risk of a serious condition being present in the embryo, and the Code fleshes out what this means.

Every centre that wants to carry out PGD needs a variation to its licence for each genetic condition it wishes to test for. There is a list of previously-approved conditions which can be authorized fairly speedily if an experienced centre wishes to add them to its licence. For new centres, and for late-onset or lower-penetrance conditions, each application must be separately approved by a licence committee.

The HFEA has also permitted preimplantation HLA-typing, provided certain criteria are met.

This paper will explore how the HFEA has made policy decisions about legitimate uses of PGD, and how, in practice, it regulates PGD. It will also consider whether the law which is currently before parliament will make any substantive changes to the regulatory framework in the UK.

**S11.1****Peroxisomal disorders: biochemistry, molecular biology and genetics****H. R. Waterham:**

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Peroxisomes are ubiquitous organelles that play an essential role in cellular metabolism as underscored by the recognition of a large number of often severe genetic disorders in which one or more peroxisomal functions are defective. These peroxisomal disorders can be classified into two main groups, including the Peroxisome Biogenesis Disorders (PBDs) and the single peroxisomal enzyme deficiencies.

The group of single peroxisomal enzyme deficiencies currently comprises 10 different defects, 5 of which involve enzymes involved in peroxisomal fatty acid beta-oxidation, including the most common peroxisomal disorder X-linked adrenoleukodystrophy (X-ALD). The distinction between the different disorders can be readily made on the basis of specific metabolite patterns in combination with selective enzyme diagnostics and followed by diagnostic DNA testing.

The PBDs comprise a group of severe, often lethal multi-systemic autosomal recessive disorders displaying considerable clinical, biochemical and genetic heterogeneity. Based on clinical and biochemical parameters, originally 3 different presentations had been defined, including Zellweger syndrome (ZS), neonatal adrenoleukodystrophy (NALD) and infantile Refsum disease (IRD), with decreasing clinical and biochemical severity. Recently, however, they have been assigned to the Zellweger Spectrum continuum based on the recognition that the different presentations can be caused by mutations in different genes, different mutations within the same gene and the fact that they show considerable clinical and biochemical overlap. A fourth entity assigned to the group of PBDs is Rhizomelic Chondrodyplasia Punctata (RCDP) type 1, the clinical presentation of which clearly differs from those observed in the Zellweger Spectrum disorders.

PBDs can be caused by mutations in any of at least 13 different *PEX* genes, which encode proteins (peroxins) involved in different stages of peroxisomal protein import and organelle biogenesis. To establish the overall mutational spectrum of PBDs with respect to the affected *PEX* gene as well as mutations therein and to allow rapid identification of the defective *PEX* gene for diagnostic purposes, we developed genetic complementation assays based on peroxisome restoration assessment following PEG-mediated fusion of patient cell lines with tester cell lines or transfection of patient cell lines with either of the known *PEX* cDNAs. Using these assays we have assigned skin fibroblasts from over 500 patients diagnosed with a PBD, to different genetic complementation groups representing defects in the various *PEX* genes. For all the genes we implemented diagnostic DNA testing involving gene sequencing.

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**S11.2****Diagnostic tools in mitochondrial respiratory chain defects****O. Elpeleg:**

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Congenital disorders of the mitochondrial respiratory chain are common inborn errors of metabolism with an incidence of 1:5,000-8,000 live births. The respiratory chain consists of 85 subunits which are assembled into five enzymatic complexes. Thirteen of the 85 subunits are encoded by the mitochondrial DNA (mtDNA) and a large number of proteins are required for the replication of the mtDNA molecule, its expression and for the assembly of each of the complexes. Most patients present with neurological symptoms accompanied by lactate elevation and the enzymatic diagnosis is established in muscle tissue. However, translation of the results of the enzymatic analysis into genetic counseling is hampered by the fact that the same defect is not present in fibroblasts and that many of the genes encoding the non-structural factors are presently unknown.

Because all our patients originate from small consanguineous families and since most of the proteins responsible for congenital disorders of the mitochondrial respiratory chain are encoded by the nuclear genome and the defects are transmitted in an autosomal recessive manner, we have used homozygosity mapping. This method, in combination with mtDNA and mtRNA quantification, resulted not only in a fast and economic diagnosis, but has also led to the identification of novel genes involved in the biogenesis of the mitochondrial respiratory chain.

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### S11.3

#### Glutaryl-CoA dehydrogenase deficiency: biochemical and molecular aspects

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Glutaric aciduria type I (GA I; OMIM 231670) is an autosomal recessive disorder due to deficient or non-functional glutaryl-CoA dehydrogenase (GCDH). The metabolic block leads to the accumulation of glutaric, and 3-hydroxyglutaric acids as well as glutarylcarnitine in body fluids. The clinical picture is characterized by the sudden onset of a severe dystonic-dyskinetic disorder, hypotonia, irritability, macrocephaly and degeneration of the caudate and putamen, which generally appear between the 5th and 14th months of age, but mild symptoms such as motor delay and hypotonia can be observed at earlier ages.

The gene GCDH consists of 11 exons and codes for a precursor protein of 438 amino acids. The active enzyme is a homotetramer. Single prevalent mutations have been found in small isolated ethnic groups. However, mutations of the GCDH gene in general population are heterogeneous. To date, more than 150 disease-causing mutations have been identified.

More than half of the reported patients had completely absence of GCDH activity, while others had a residual activity between 5- 15% and very few patients had a residual activity up to 30%. Complete absence of activity was connected to certain mutations. The most frequent was p.R402W. Less but also frequent was the mutation p.A293T. This group of patients all excreted considerable amounts of both glutarate and  $\beta$ -hydroxyglutarate with significant higher amounts of the former. The most frequent mutations associated with residual activity, were p.V400M, p.R227P and p.A421V and most of these patients had low excretion of glutarate and  $\beta$ -hydroxyglutarate with highest excretion of the latter compound. Therefore, it seems clear that two distinct genetically and biochemically groups of patients exist, but the severity of the clinical phenotype seems to be closely linked to the development of encephalopathic crisis rather than to residual enzyme activity or to the genotype.

### S12.1

#### Genetical genomics: a combination of genetic variation with genomic profiling to reconstruct molecular networks

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Genetically different individuals can exhibit large quantitative variation for disease susceptibility, physiology, and many other traits of interest. Such variation stems, at least partly, from variations in the DNA. Modern sequencing technologies can reveal the variations in the (epi)genome, and genome-wide linkage (GWL) analysis and genome-wide association (GWA) analysis can then link or associate them to variations in the trait of interest. These strategies are increasingly applied to a growing number of organisms, including human, mouse, rat, cattle, pigs, *A. thaliana*, tomato, corn, yeast, *C. elegans*, and *D. melanogaster*, and have pinpointed many quantitative trait loci (QTL) on the genome. To lift the veil that covers the genome-to-phenotype relation we may need to monitor the whole trajectory of intermediate biomolecular phenotypes. Today's molecular technologies, particular microarray and deep sequencing for transcriptome and high resolution mass spectrometry and nuclear magnetic resonance for proteomics and metabolomics, have reached a cost-efficiency level allowing for comprehensive molecular profiling of many samples at multiple biomolecular levels. We here discuss the results, promises and pitfalls for network reconstruction using system-wide data on gene expression (eQTLs), proteins (pQTLs) and metabolites (mQTLs) from studies on human, mouse and *A. thaliana*.

### S12.2

#### Metabolic networks in biology and diseases

##### Z. Oltvai;

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Most diseases are the consequence of the breakdown of cellular processes, but the relationships among specific genetic/ epigenetic defects and variations (SNPs, CNVs, etc) and these disruptions, the molecular interaction networks underlying them, and the disease phenotypes remains poorly understood. To begin to gain insights into such relationships we have constructed a bipartite human disease association network in which nodes are diseases and two diseases are linked if mutated enzymes associated with them catalyze adjacent metabolic reactions. We will describe the characteristics of this network and show that the structure and modeled function of the human metabolic network can provide insights into disease comorbidity, with potentially important consequences for disease diagnosis and prevention.

### S12.3

#### Reverse engineering the transcriptional networks

##### D. diBernardo;

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One of the main challenges in the era of post-genomic research is to develop methods to extract information from the vast amount of data generated by high-throughput techniques. Tools such as microarrays probing expression of all known genes are now a standard technique worldwide, whereas new approaches based on sequencing are fast emerging.

This genome-wide data yields information not only at the single gene level, but also at the 'systems' level, i.e. how genes, proteins and metabolites interact with each other to perform a specific function. In order to 'read' such information new methods coming from quantitative sciences such as physics and engineering, have to be used.

We will introduce experimental protocols and computational algorithms to infer gene regulatory networks.

We have applied our reverse-engineering approach to elucidate the transcriptional network regulated by the transcription factor p63, whose mutations are causative of human malformation syndromes, in primary keratinocytes.

We have identified over 100 novel targets of p63 and shown that it transiently regulates members of the AP-1 protein complex.

**S13.1****Dysregulated RAS signaling in Noonan syndrome and related disorders****M. Tartaglia:***Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy.*

In the last few years, mutations in genes coding for transducers with role in the RAS-MAPK signalling pathway have been identified as the molecular cause underlying a group of clinically related developmental disorders with features including reduced postnatal growth, facial dysmorphia, cardiac defects, ectodermal anomalies, cognitive deficits and variable predisposition to certain malignancies. Noonan syndrome (NS), which is the most common condition among these Mendelian traits, is caused by heterozygous mutations in *PTPN11*, *SOS1*, *KRAS* and *RAF1* in approximately 65% of affected individuals. Missense *PTPN11* and *RAF1* mutations also account for the vast majority of LEOPARD syndrome (LS), while defects in *KRAS*, *BRAF*, *MEK1* and *MEK2*, and a bunch of missense changes in *HRAS* occur in 60-80% of cardiofaciocutaneous syndrome (CFCS) and in Costello syndrome (CS), respectively.

The RAS-MAPK signalling pathway controls cell proliferation, survival and differentiation, and represents the most common target for somatic activating mutations in cancer. NS-, LS-, CFCS- and CS-causing alleles encode for proteins with aberrant biochemical and functional properties, mostly resulting from impaired catalytic autoinhibition, that promote increased signal flow through the MAPK cascade.

The available structural, molecular and biochemical data support the view that, besides its crucial role in oncogenesis, dysregulation of RAS-MAPK signalling has profound consequences on development. These findings also provide evidence that germline transmitted mutations causing developmental disorders define a novel allele series that have distinctive perturbing role on signaling, and offer a model in which distinct gain-of-function thresholds of the activity of individual transducers are required to induce cell-, tissue- or developmental-specific phenotypes, each depending on the transduction network context involved in the phenotype.

**S13.2****The molecular dissection of Joubert syndrome and allied ciliopathies**

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Joubert syndrome is a disorder that is primarily characterised by a typical hind brain malformation known as the "molar tooth sign", as seen on CT and MRI images. Retinal degeneration, cystic kidneys, mental retardation and abnormal breathing patterns (episodic hypernea and apnea) are also common features. To date, four genes are known to be involved in Joubert syndrome, and three of the protein products localize to primary cilia.

We examined the function of the RPGRIP1-like protein (RPGRIP1L), encoded by *RPGRIP1L* on chromosome 16q12.2. This is the homologue of RPGRIP1 (RPGR interacting protein 1), a ciliary protein involved in congenital blindness (Leber congenital amaurosis). *RPGRIP1L* is ubiquitously expressed and its protein product localizes to basal bodies of cilia in brain, retina and kidney. We identified homozygous frameshift and splice site mutations in two families with typical Joubert syndrome and compound heterozygous nonsense and missense mutations in a third family. All mutations disrupt the interaction of the C2-domain of RPGRIP1L with nephrocystin-4, encoded by *NPHP4* which is mutated in Senior Løken syndrome patients (retinal dystrophy and cystic kidneys). Interestingly, one of the patients had post-axial polydactyly and encephalocele resembling the Meckel-Gruber syndrome phenotype, suggesting that *RPGRIP1L* could be involved in disorders that represent different spectra of the same underlying defect.

We and others have found that these disorders with overlapping phenotypes in the retina, the brain and the kidney, can result from perturbation of individual components of shared functional modules, regulating distinct processes that are based at (primary) cilia. Functional

dissection of these modules has yielded important information of the molecular pathogenesis of the associated disorders, the "ciliopathies" disease family. They point towards a role of the cilia in regulating a wide variety of basic cellular processes, such as vesicle transport, Wnt signalling and Hedgehog signalling. Furthermore, similar to the identification of *RPGRIP1L*, they have provided us with a valuable collection of novel "ciliopathy candidate genes", that will even expand the ciliary factor in various disease processes.

**S13.3****Alterations of FGF signalling in LADD syndrome****B. Wollnik:***Institute of Human Genetics, Cologne, Germany.*

Mutations in different components of the FGF signalling pathway cause the lacrimo-auriculo-dento-digital (LADD) syndrome, an autosomal dominant disorder mainly characterized by anomalies of the lacrimal system, ears and hearing, teeth, and distal limb development. Notably, all LADD mutations identified so far in FGFR2/3 are located within the tyrosine kinase (TK) domains of the receptors within loops that play an important regulatory function in the control of receptor activity. Our functional studies of FGFR2 LADD mutants indicated a reduced tyrosine kinase activity of the receptor itself as well as reduced FGFR2-mediated substrate phosphorylation and reduced downstream signalling. Moreover, the timely and precisely ordered dynamics and patterns of autoposphorylation are changed in FGFR2 mutants as shown by biochemical investigations and crystal structure analysis. While FGFR2 LADD mutants exert a putative dominant-negative effect on normal FGFR2 protein, FGF10 LADD mutations cause haploinsufficiency. Beside novel mutational mechanisms of known LADD genes, we found an autosomal recessive inheritance in a severely affected LADD patient caused by the homozygous p.R579W in the TK domain of FGFR2. Interestingly, we also observed molecular overlaps of LADD-like phenotypes with p63-related disorders. Our data shed light on pathophysiological mechanisms underlying LADD syndrome and expand the spectrum of disorders associated with altered FGF signalling.

**S14.1****Molecular mechanisms of cellular senescence****F. Fagagna:***IFOM-IEO Campus, Milan, Italy.*

Early tumorigenesis is associated with the engagement of the DNA-damage checkpoint response (DDR). Cell proliferation and transformation induced by oncogene activation are restrained by cellular senescence. It is unclear whether DDR activation and oncogene-induced senescence (OIS) are causally linked. Here we show that the expression of an activated oncogene (H-RasV12) in normal human cells, results in a permanent cell cycle arrest caused by the activation of a robust DDR. Experimental inactivation of DDR abrogates OIS and promotes cell transformation. DDR and OIS are established after a hyper-replicative phase occurring immediately after oncogene expression. Senescent cells arrest with partly replicated DNA and with DNA replication origins having fired multiple times. In vivo DNA labelling and molecular DNA combing reveal that oncogene activation leads to augmented numbers of active replicons and to alterations in DNA replication fork progression. Therefore OIS results from the enforcement of a DDR triggered by oncogene-induced DNA hyper-replication. Senescence is also associated with a global heterochromatinization of nuclear DNA. These senescence associated heterochromatic foci (SAHF) are enriched in histone H3 di-tri methylated on lysine 9 (H3K9m) and HP1 proteins and High mobility group A (HMGA) proteins are also known to be essential structural components of SAHF. Our most recent results on the interplay between DDR activation and oncogene-induced heterochromatinization will be presented.

**S14.2****Cornelia de Lange Syndrome and the Cohesinopathies: Developmental Repercussions of Cohesin Dysfunction****I. D. Krantz:***Division of Human Genetics and Molecular Biology, The Children's Hospital of Philadelphia, Philadelphia, PA, United States.*

The cohesin proteins compose an evolutionarily conserved complex whose fundamental role in chromosomal cohesion and coordinated

segregation of sister chromatids has been well characterized across species. Recently regulators and structural components of cohesin have been found to surprisingly cause specific human developmental disorders (collectively termed "cohesinopathies") when mutated. Mutations in NIPBL, the vertebrate homolog of the yeast Sister chromatid cohesion 2 (Scc2) protein, a regulator of cohesin loading and unloading, are responsible for approximately 50% of cases of Cornelia de Lange syndrome (CdLS). Mutations in the cohesin structural components SMC1A and SMC3 were also found to result in CdLS. CdLS is a multisystem developmental disorder classically characterized by facial dysmorphia, upper extremity malformations, hirsutism, cardiac defects, growth and cognitive retardation, and gastrointestinal abnormalities. A mild form of CdLS has been consistently reported, however, it had not been clear if this is a distinct etiologic entity from classic CdLS or truly a mild manifestation, however molecular testing of cohesin genes has identified mutations in individuals with very subtle features of CdLS bordering on apparent isolated mental retardation. Mutations in another cohesin regulator, ESCO2, result in Roberts syndrome (RBS) and SC phocomelia. Roberts syndrome is a recessively inherited multisystem disorder with craniofacial, limb, cardiac, other systemic abnormalities and neurocognitive dysfunction. While there is some overlap between Roberts syndrome and CdLS they are clinically readily differentiated. Other developmental disorders have also recently been found to be associated with cohesin dysfunction. The recent implication of the cohesin complex and its regulators in transcriptional control has shed light on the mechanism by which alterations in this complex leads to the specific phenotypes seen in these disorders. A review of cohesin function, the disorders associated with disruption of this pathway and future clinical and bench-top research directions will be discussed.

#### S14.3

#### Nijmegen breakage syndrome: clinical manifestation of defective response to DNA double-strand breaks

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Patients with the human genetic disorder, Nijmegen Breakage Syndrome (NBS) display a characteristic facial appearance, microcephaly and a range of symptoms including immunodeficiency, growth retardation and chromosomal instability. NBS patients have an extremely high risk of developing lymphoma. Patients were found to be highly sensitive to ionising irradiation (IR) and this radiosensitivity had fatal consequences in some undiagnosed patients. The most dangerous DNA-lesion caused by IR is considered to be the double-strand break (DSB) and indeed, NBS patient cells are sensitive to all mutagens which produce DSBs directly or indirectly.

The underlying gene, *NBN*, codes for a protein, nibrin, involved on the one hand, as a "caretaker", in the processing/repair of DNA double strand breaks and on the other hand, as a "gatekeeper", in the regulation of cell cycle checkpoints. The majority of patients are homozygous for a founder mutation in *NBN*, a 5bp deletion in exon 6. This mutation leads to a truncated amino-terminal fragment containing FHA and BRCT domains, and a carboxy-terminal protein (p70-nibrin) which is produced by alternative initiation of translation from a cryptic upstream start. *NBN* is an essential gene and it is clear that the carboxyterminal p70-nibrin protein is sufficient to ensure patient survival.

We have been examining patient cells and conditional *Nbn* null mutant mouse cells in order to establish which functions of full length nibrin can be carried out by the carboxy terminal fragment and, more particularly, how this partial functioning explains aspects of the human disease. In this connection a further rare nibrin fragment, p80-nibrin, which is associated with a milder course of the disease has been of particular interest since it may define the basis of a potential anti-cancer prophylactic treatment for NBS patients.

#### S15.1

#### Genomic Perspectives on Human Origins

*S. Pääbo;*

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One approach to understanding what makes humans unique as a species is to perform structural and functional comparisons between the genomes of humans and our closest evolutionary relatives the great

apes. Recently, the draft sequences of the chimpanzee and rhesus macaque genomes have opened up new possibilities in this area. I will discuss work that compares functional and structural aspects of the human and ape genes, using FOXP2, a gene involved in speech and language, as an example. I will also discuss how a genome-wide analysis of the Neandertal genome will enhance our ability to identify genes that have been of importance during human evolution.

#### S15.2

#### Human genetic population structure: Patterns and underlying processes

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Classical studies of genetic diversity in humans consistently showed that the largest proportion of human diversity occurs among members of the same population. On average, differences among different populations in the same continent represent 5% of the global human variance, and differences among continents another 10%. Genetic variation is largely discordant across the genome, meaning that different loci show different spatial patterns, and implying that a good description of population structure can only be based on the analysis of multiple loci. Studies of single loci are also unlikely to reasonably identify an individual's place of origin. A general decline of genetic diversity with distance from Africa, and a parallel increase in linkage disequilibrium, can be accounted for by the effects of a series of founder effects accompanying the spread of anatomically-modern humans from Africa. Recent DNA analyses at the global level show that most allelic variants are cosmopolitan and only a small percentage are continent-specific, whereas a clearer continental structure emerges when considering composite haplotypes. This suggests that, at the global level, gene flow has had a strong impact on genetic diversity, through both directional dispersal and successive short-range migratory exchanges. At the local level, several factors have contributed to genetic differentiation, and, in particular, language barriers have been shown to be associated with small but non-negligible increases of the genetic differences between neighboring populations.

#### S15.3

#### From genetic diversity to the understanding of basic biological function: towards evolutionary systems biology

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## ESHG CONCURRENT SESSIONS

### C01.1

#### Clinical and molecular characteristics of 1qter syndrome: Delineating a critical region for corpus callosum agenesis/hypogenesis

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Patients with a microscopically visible deletion of the distal part of the long arm of chromosome 1 have a recognisable phenotype, including mental retardation, microcephaly, growth retardation, a distinct facial appearance and various midline defects including corpus callosum abnormalities, cardiac, gastro-oesophageal and urogenital defects as well as various central nervous system anomalies.

So far, only 8 cases with a pure submicroscopic deletion of distal 1q have been clinically described. In general, patients with a submicroscopic deletion have a similar phenotype, suggesting that the main phenotype of these patients is caused by haploinsufficiency of genes in this region.

In the present study we describe the clinical presentation of 13 new patients with a submicroscopic deletion of chromosome 1q43q44, of which 9 were interstitial, and report on the molecular characterisation of the deletion size.

The clinical presentation of these patients has clear similarities with previously reported cases with a terminal 1q deletion. Corpus callosum abnormalities were present in ten of our patients. The AKT3 gene has been reported as an important candidate gene causing this abnormality. However, through detailed molecular analysis of the deletion size in our patient cohort, we were able to delineate the critical region for corpus callosum abnormalities to a 360 kb genomic segment which contains four possible candidate genes, but excluding the AKT3 gene.

### C01.2

#### Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation

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Submicroscopic copy number imbalances contribute significantly to the genetic etiology of human disease. We report on a novel micro-duplication hot spot at Xp11.22 identified in 6 unrelated families with predominantly nonsyndromic XLMR. All duplications are unique and segregate with the disease, including the large families MRX17 and MRX31. Our FISH data are strongly suggestive for tandem duplication events with their sizes ranging from 0.4 to 1.0 Mb with a minimal, commonly duplicated region that contains three genes: RIBC1, HSD17B10 and HUWE1. RIBC1 could be excluded based on its absence of expression in the brain and since it escapes X-inactivation in females. For the other genes, expression array and quantitative PCR analysis in patient cell lines compared to controls showed a significant up-regulation of HSD17B10 and HUWE1 as well as several important genes in their molecular pathways. Loss-of-function mutations of HSD17B10 have previously been associated with progressive neurological disease and XLMR. The E3 ubiquitin ligase HUWE1 has been implicated in TP53-associated regulation of the neuronal cell cycle. We also detected segregating sequence changes of highly conserved residues in HUWE1 in three XLMR families, which are possibly associated with the phenotype. Mutations in HSD17B10 have previously been reported to be associated with XLMR and a progressive neurological disorder. Our findings demonstrate that an increased gene dosage of HSD17B10, HUWE1, or both contribute to the etiology of XLMR, and suggest that point mutations in both genes are associated with this disease too.

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### C01.3

#### Clinical outcome and molecular investigation of Pitt-Hopkins syndrome: a series of 9 patients

**L. de Pontual, Y. Mathieu, M. Rio, A. Munich, S. Lyonnet, J. Amiel;**

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Pitt-Hopkins syndrome (PHS) is a syndromic encephalopathy characterised by severe psychomotor delay, epilepsy, daily bouts of diurnal hyperventilation starting in infancy, and distinctive facial features. A systematic 1Mb resolution genome wide BAC array identified a 1.8 Mb *de novo* microdeletion on chromosome 18q21.1 in 1 case. We subsequently identified *de novo* heterozygous mutations of TCF4 gene in 8 additional PHS cases. These findings provide the first evidence of a human disorder related to class I basic helix-loop-helix transcription factor (also known as E-proteins) defects. Our data support that haploinsufficiency is the most likely disease-causing mechanism, while a dominant-negative effect is an alternative hypothesis currently being tested for missense mutations occurring in the basic domain. Expression analysis of the TCF4 gene during human embryonic development will also be presented.

Bouts of hyperventilation and epilepsy, although distinctive, are not fully penetrant. Several clinical features will be underscored as possible diagnostic clues in particular the facial gestalt, dysautonomia and subtle immunoglobulin deficiency. EEG and brain MRI may also give valuable clues that will be discussed. Patients diagnosed with PHS display a broad spectrum of dysautonomic features that will be detailed. These data may also shed new light on the normal processes underlying autonomic nervous system development and maintenance of an appropriate ventilatory neuronal circuitry.

### C01.4

#### Expanding the clinical phenotype of tetrasomy 18p

**C. D. Sebold<sup>1</sup>, E. Roeder<sup>1,2</sup>, B. T. Soileau<sup>1</sup>, A. Malik<sup>1</sup>, D. Neigut<sup>2</sup>, K. Hernandez<sup>3</sup>, M. Thomas<sup>3</sup>, B. Perry<sup>4</sup>, P. Fox<sup>5</sup>, M. Semrud-Clikeman<sup>6</sup>, B. Butcher<sup>6</sup>, S. Smith<sup>7</sup>, L. O'Donnell<sup>1</sup>, K. Richards<sup>8</sup>, K. Reinker<sup>9</sup>, R. Tragus<sup>1</sup>, D. E. Hale<sup>1</sup>, J. D. Cody<sup>1</sup>**

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**Background.** Thus far, the phenotype of tetrasomy 18p has been primarily delineated by a series of published case series and case reports. Findings reported in more than 25% of these cases include neonatal feeding problems, growth retardation, microcephaly, strabismus, abnormalities in muscle tone, scoliosis/kyphosis, and variants on MRI. Developmental delays and mental retardation are also universally present. **Methods.** To further refine the phenotype and natural history of tetrasomy 18p, we reviewed the medical history and records of 34 individuals with tetrasomy 18p. In addition, 20 individuals with tetrasomy 18p were clinically evaluated at our center. These individuals had multiple evaluations, including endocrinology, ophthalmology, neuropsychology, orthopedics, ENT, and genetics. They also underwent an MRI as well as a hearing test. **Results.** As a result of these analyses,

we can expand the phenotypic description of tetrasomy 18p. Findings identified in more than 25% of our patient population included neonatal jaundice, recurrent otitis media, hearing loss, seizures, refractive errors, a history of constipation and gastroesophageal reflux, heart defects, pes planus, and attention problems. Dysmorphic features that were reported in more than 25% of the population included ptosis; posteriorly rotated ears; unraveled helices; small ears; abnormal columella; smooth philtrum; small mouth; thin upper lip; abnormal Cupid's bow; palatal abnormalities, and a prominent or pointed chin. **Conclusion.** These findings further our knowledge of the clinical manifestations of tetrasomy 18p and will enable physicians to better anticipate and manage complications as they arise.

#### C01.5

##### **Congenital nephrotic syndrome, microcephaly, trigonocephaly, polydactyly, brain and eye anomalies: a distinct autosomal recessive disorder**

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Congenital nephrotic syndrome (CNS) is a heterogeneous disease. While genetic causes of isolated CNS are well established, the knowledge on the genetic basis of various syndromic forms is still fragmentary. One of them is characterized by the association of CNS with microcephaly / brain anomalies, also known as Galloway-Mowat syndrome (GMS). It is obvious that GMS itself is not a homogeneous entity.

We observed a strikingly similar form of syndromic CNS in four children originating from unrelated consanguineous families. All affected children had gross proteinuria from birth and rapid progress to end stage renal failure. Two had evidence of additional tubular involvement. Head circumference fell below the 3rd centile within the first months of life in all patients. Trigonocephalic head shape was present in three of them. All children had cerebral gyration anomalies, and Dandy-Walker malformation was present in two. Further constant findings were postaxial hexadactyly and eye anomalies including iris atrophy, non-reactive miosis, coloboma, and microphthalmia. Three children had an atrial septal defect.

One of these patients was reported previously (Mildenberger et al.: Acta Paediatr 1998), but no other similar reports exist to our knowledge. We propose that this is a distinct autosomal recessive disorder within the heterogeneous group of microcephaly-nephrosis syndromes. It may be considered a subtype of GMS or a separate entity (Mildenberger syndrome). There is an obvious overlap with Pierson syndrome (OMIM 609049), which is caused by LAMB2 mutations, but no mutations in this gene were found in the disorder presented here.

#### C01.6

##### **An undescribed phenotype associated with crano-fronto-facio-nasal malformations, total alopecia and genital abnormalities**

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We report an unusual malformation in the cranofrontonasal region of four males from two distinct inbred families. Major characteristics are; 1-) total alopecia; 2-) brachycephaly due to coronal suture synostosis; 3-) frontonasal dysostosis providing hypertelorism, blepharophimosis, severely depressed nasal bridge with bifid tips; 4-) posterior cranial skull defects; 5-) small naevi on the posterior skull; 6-) rotatory nystagmus and 7-) corpus callosum agenesis. These malformations are also associated with bilateral cryptorchism and hypogonadism. The first family contains three affected individuals with one premature death at the age of two months, the others, 13 and 45 years old respectively, belong to different branches of the same family. The second pedigree contains a three months old single affected male with similar symp-

toms. Since both families originated from nearby cities in the Blacksea region of Turkey, this suggested a possible role of a founder mutation. As a consequence of the highly inbred nature of these families an autosomal recessive mode of inheritance is likely. Alternatively, since all patients are males, X-linked inheritance challenged by inbreeding should also be considered. Some of these findings apparently overlap with cerebrofrontofacial (OMIM 6085789) and craniofrontonasal (OMIM 304110) syndromes. However, neither a total alopecia, nor hypogonadism has been associated with the aforementioned conditions previously. To the best of our knowledge, this is a new disorder with severe crano-fronto-nasal malformations in association with alopecia and genital abnormalities. Clinical and molecular investigations of this new disorder are done within the CRANIRARE consortium supported by the European Research Area Network "E-RARE".

#### C02.1

##### **Fas-associated factor-1, a protein involved in apoptosis, causes cleft lip and palate**

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Cleft lip and palate is the most common craniofacial birth defect with complex etiology. We show that a reciprocal translocation, which cosegregates with cleft palate in a family, disrupts the Fas-Associated Factor-1 (FAF1) gene. The mutation results in lowered expression, and likely haploinsufficiency. Transmission disequilibrium analysis demonstrates that FAF1 associates with cleft lip and palate. In situ hybridization unravels high levels of mFaf1 along the lips and the medial edge epithelium (MEE) of the fusing palate in mice. Moreover, in zebrafish larvae, zFaf1 is mostly expressed in the pharyngeal cartilages, where its knock-down results in orofacial defects. As FAF1 is a member of the Fas death-inducing complex that initiates apoptosis, it likely causes cleft palate by preventing MEE degeneration. The data provides strong molecular evidence that "death", rather than epithelial mesenchymal transformation or anterior/posterior migration, is the major fate for MEE. These data also predict that other factors in the FAS-induced apoptosis pathway likely play a role in cleft pathogenesis.

#### C02.2

##### **Sporadic venous malformation is caused by somatic mutations in TIE2**

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Venous malformations (VM) are the most frequent vascular malformations referred to vascular anomaly centers. An autosomal dominant familial form, termed cutaneomucosal venous malformation (VMCM), representing about 1% of venous lesions, is caused by gain-of-function mutations in the TIE2 gene. The aetiology of sporadic VM, which represents more than 95% of venous lesions, has however remained unknown. Here we show that sporadic VMs are caused by somatic mutations in TIE2. We identified seven missense mutations in VM tissue-derived DNA, which were however absent in blood DNA from these patients, and in tissue DNA from 90 controls. All of the mutations, predicted by bioinformatic analysis to have deleterious effects of varying severity on protein function, were found to result in a strong in vitro ligand-independent increase in phosphorylation of TIE2. In some patients, we observed two mutations acting in cis. Such combinations on the same allele induced even higher phosphorylation levels of the

receptor than the constituent single mutations. Additional mutations were identified in lesion-derived cDNA, suggesting that the number of cells carrying a mutation can be in the minority, making the reduction of tissue heterogeneity an important factor in mutation detection. These data provide molecular evidence that sporadic venous malformations are caused by somatic activating TIE2 mutations, which are often linked to additional somatic genetic events. They explain the localized, predominantly unifocal nature of these lesions, and furthermore, pinpoint the TIE2 signaling pathway as a target for the development of therapeutic interventions. (miikka.vikkula@uclouvain.be)

### C02.3

#### ***LTBP2 mutation in autosomal recessive microspherophakia with some marfanoid features***

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Microspherophakia is a lens malformation encountered in Marfan (MFS) and Weill-Marchesani (WMS) syndromes. We observed a large consanguineous family with three children affected with microspherophakia. The proband had tall stature with an arm span larger than his height, long slender fingers, and a high-arched palate. He did not meet the diagnostic criteria for MFS, nor WMS. No mutation was found in the MFS-associated gene *FBN1* (CMG, University of Gent, Belgium). We mapped the locus by homozygosity to a 12.6 cM region of chromosome 14q2 using a 10K GeneChip SNP array in the affected siblings, followed by microsatellite analysis, with a multipoint LOD of 2.57. The linkage interval contained one conspicuous candidate gene, *LTBP2*, encoding a latent transforming growth factor-beta binding protein. LT-BPs are extracellular matrix proteins with multiple domain structures bearing strong homologies with fibrillins, and may play several roles, including finely controlling TGF-β activity in the matrix, a structural role in microfibrils, and a role in cell adhesion. We found a truncating mutation g.76339dupC (p.Pro599ProfsX4), homozygous in the affected siblings, heterozygous in the parents, and absent from 100 unrelated control subjects from the same ethnic group. Using a polyclonal antibody, we found *LTBP2* to be strongly expressed in the calf ciliary zonule. Fibroblast cultures and lymphoblast cell lines from the affected siblings are under study. We conclude that the *LTBP2* truncating mutation reported here is a rare cause of microspherophakia with marfanoid features.

### C02.4

#### ***Novel ARVD5 gene causes autosomal dominant sudden cardiac death due to missense mutations in the TMEM43 gene***

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Autosomal dominant arrhythmogenic right ventricular cardiomyopathy/dysplasia (ARVC/D) causes sudden cardiac death and is characterized by clinical and genetic heterogeneity. Fifteen unrelated ARVC families from Newfoundland's founder population were identified wherein patients with ARVC shared an ancestral haplotype on chromosome 3p (*ARVD5*). Identification of key recombination events and sequencing of the 20 annotated genes mapping within the *ARVD5* critical region identified one rare variant that resulted in a missense mutation in all patients from all families in Transmembrane Protein 43 (*TMEM43* 1073 C>T, S358L). This variant was not found in population-matched controls. Interestingly, *TMEM43* is not predicted to be a desmosomal protein. Although little is known about the function of the *TMEM43* protein in the cardiac myocyte, the gene contains a response element for PPARγ (an adipogenic factor) which may explain the fibrofatty replacement of the myocardium, a characteristic pathological finding in ARVC, and may act upstream of the desmosomal proteins that cause other forms of ARVC. Results from full gene sequencing of *TMEM43* in a series of 150 ARVC probands from the UK will also be presented.

### C02.5

#### ***Knock-out models in mice and men suggest a proatherogenic role for USF1***

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Disturbances in body lipid homeostasis are tightly linked with cardiovascular disease (CVD). We recently established the association of USF1 transcription factor with high blood triglycerides and cholesterol (Pajukanta et al, 2004).

We have generated a strain of *Usf1* knockout (-/-) mice which along with their +/- and +/+ littermates (n=12) were fed with 'Western' diet rich in triglycerides (TG) and cholesterol for 8 weeks. After the diet *Usf1* -/- mice had significantly lower blood (p<0.05) and VLDL (p<0.01) TG than their +/+ littermates. By using Affymetrix expression arrays, we observed that lipoprotein lipase (Lpl) mRNA levels were elevated (p<0.01) in -/- mice liver and muscle as compared to controls, consistent with the TG phenotype. In -/- mice adipose tissue, the cholesterol biosynthetic pathway was significantly down-regulated when compared to their +/+ littermates.

We also established a cellular model representing USF1 knock-down, employing treatment of human hepatoma cells (HuH7) with USF1 siRNAs. We labeled the cells for 30 min with [<sup>3</sup>H]acetic acid, followed by a chase, and quantified the [<sup>3</sup>H]cholesterol and TG synthesized. A decreased amount of both [<sup>3</sup>H]cholesterol and TG (p<0.05) was observed in siRNA treated cells as compared to controls. Again, with Affymetrix microarrays we observed that several cholesterol biosynthetic pathway enzymes were down-regulated in USF1 knockdown cells. Since the loss-of-function of USF1 caused a decrease in plasma TG in vivo as well as cholesterol and TG biosynthesis in vitro, we hypothesize that USF1 could be proatherogenic and its knockdown in appropriate tissues actually has a favorable cardiovascular effect.

### C02.6

#### ***Disruptions of highly conserved, very distant regulatory elements on either side of SOX9 are associated with Pierre Robin sequence***

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Most evolutionarily conserved human DNA has no protein-coding function but may have regulatory function. The mechanism of action and size of the domains controlled by these highly conserved non-coding elements (HCNE) remains largely unknown. We show here that disruption of very distant cis-regulatory HCNE on either side of SOX9 gene causes Pierre Robin sequence (PRS), a common and important orofacial cleft anomaly with hypoplastic jaw. Evidence for an autosomal dominant PRS locus on 17q24 comes from linkage in a large PRS family and a cluster of breakpoints in three independent familial translocations mapping to gene desert 1.06-1.23 Mb centromeric to SOX9. We identified potential regulatory mutations via comparative genomic hybridization across a 3.6Mb conserved region surrounding SOX9. Heterozygous microdeletions involving one or more HCNEs were identified in three of eleven PRS patients 1.38-1.58 Mb up- or downstream of the SOX9 gene. In one family a single non-polymorphic nucleotide change in a HCNE was found that altered transcription factor binding and enhancer function in vitro. Interphase FISH analysis of the orthologous region in 13.5 dpc mouse embryos showed that this

normally condensed genomic region demonstrates dynamic expression-dependent chromatin decondensation in the developing mandible. Normal development of the mandible thus requires the action of very long-distance cis-acting elements operating on both sides of the SOX9 promotor, at transcriptional and chromatin levels. The domain of action of tissue-specific cis-regulation appears to be very large indeed and this has significant implications for mutation analysis in human disease and genome biology

### C03.1

#### Mitochondrial complex 3 deficiency associated with homozygous mutation in *UQCRCQ*, encoding ubiquinol - cytochrome c reductase, complex III subunit VII, 9.5kDa

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A consanguineous Israeli Bedouin kindred presented with an autosomal recessive phenotype of severe psychomotor retardation and extrapyramidal signs, dystonia, athetosis and ataxia, mild axial hypotonia, marked global dementia with defects in verbal and expressive communication skills. Metabolic workup was normal except for mildly elevated blood lactate levels. Brain MRI showed increased density in the putamen, with decreased density and size of the caudate and lentiform nuclei. Reduced activity specifically of mitochondrial complex 3 was evident in muscle biopsies. Homozygosity of affected individuals to *UQCRCB* and to *BCS1L*, previously associated with isolated complex 3 deficiency, was ruled out. Genomewide linkage analysis identified a homozygosity locus of ~9cM on chromosome 5q31 that was further narrowed down to 2.14cM, harboring 30 genes (LOD score 8.82 at θ=0). All 30 genes were sequenced, revealing a single missense (Ser45Phe) mutation in *UQCRCQ* (ubiquinol - cytochrome c reductase, complex III subunit VII, 9.5kDa), one of the 10 nuclear genes encoding proteins of mitochondrial complex 3.

### C03.2

#### Genetic defects underlying autosomal recessive nonsyndromic hearing impairment in Turkey; three novel and five known genes

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Autosomal recessive nonsyndromic hearing impairment (ARNSHI) is genetically highly heterogeneous. To date, about 70 loci for ARNSHI have been mapped; for 26 of these loci the causative gene has been identified.

Ninety-four Turkish families with ARNSHI were evaluated. Screening of the *GJB2* gene revealed mutations in 29 of the families. In a number of *GJB2*-negative families genome-wide homozygosity mapping was performed and in twelve of these families causative mutations were found in *TMC1*, *TMPRSS3*, *DFNB59*, *MYO15A* and *LHFPL5* (*TMHS*). The latter was novel. Four families were found to have syndromic forms of hearing loss with mutations in *SLC26A4* or *USH1G*.

In family TR21 the linkage interval partially overlapped with the previously described DFNB35 locus. Sequencing of candidate genes in the overlapping region revealed a mutation in *ESRRB*. Screening of *ESRRB* in the original DFNB35 family and in three other Pakistani DFNB35-linked families revealed four additional mutations. RNA *in situ* hybridization and immunohistochemical analyses indicate that *ESRRB* is essential for inner ear development and ion homeostasis.

In family TR56 a novel locus (DFNB63) was found. Combination of results which we obtained with the separately described DFNB63 families narrowed the critical interval. Sequencing of the genes from this interval revealed four mutations in the *DFNB63* gene. *In situ* hybridization and RT-PCR analyses show expression of the *DFNB63* gene in inner ear and some other tissues.

Our results indicate that in 43 (48%) of the 90 ARNSHL families causative mutations distributed in eight different genes, of which *LHFPL5*, *ESRRB* and *DFNB63* were novel.

### C03.3

#### Thromboxane synthase mutations in an increased bone density disorder (Ghosal syndrome)

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Ghosal hematodiaphyseal dysplasia syndrome (GHDD) is a rare autosomal recessive disorder characterized by increased bone density with diaphyseal involvement, abnormal long bone modeling and cortical hyperostosis associated with aregenerative corticosensitive anemia and chronic inflammation. Studying four consanguineous GHDD families, we first localized the disease locus gene on chromosome 7q33-q34 and then identified four distinct homozygous mutations in the thromboxane synthase gene (*TBXAS1*) which codes for thromboxane synthase (TXAS). The mutations segregated with the disease and were not identified in 210 chromosome controls. TXAS is an enzyme of the arachidonic acid (AA) cascade and converts prostaglandins H<sub>2</sub> into Thromboxane A<sub>2</sub> (TXA<sub>2</sub>), which is a powerful inducer of platelet aggregation. We therefore investigated primary haemostasis in our subjects. No history of spontaneous bleeding disorder was reported but platelet studies from GHDD patients revealed a specific deficit in AA aggregation and platelet exocytosis. In addition, ELISAs detecting TXB<sub>2</sub>—the metabolite of TXA<sub>2</sub>—and PGE<sub>2</sub> showed low TXB<sub>2</sub> and high PGE<sub>2</sub> levels in patients, which might be responsible for anemia and inflammation observed in GHDD.

Finally, in order to elucidate the mechanism of increase bone density in GHDD, we investigated the effect of TXAS and TXA<sub>2</sub> on *RANKL* and *OPG* expression in primary cultured osteoblasts and found that adding a stable analog of TXA<sub>2</sub> markedly increased *RANKL* and decreased *OPG* while the addition of a specific inhibitor of TXAS had an opposite effect. These findings suggest that thromboxane synthase acts as a local regulator of bone resorption with a key function in bone remodeling.

### C03.4

#### Congenital arthrogryposis: autosomal recessive lethal congenital contractual syndrome caused by mutations in *PIP5K1C* and in *ERBB3*

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We demonstrate that mutations in genes associated with the phosphatidylinositol pathway cause lethal congenital contractual syndrome (LCCS), a neurogenic form of arthrogryposis. We previously mapped

an autosomal recessive form of the disease (LCCS2, MIM 607598) in Israeli Bedouin kindred to chromosome 12q13. We now mapped a similar phenotype (LCCS3) in another Bedouin kindred to 3.4Mb on chromosome 19p13, demonstrating a homozygous mutation in *PIP5K1C*, encoding phosphatidylinositol-4-phosphate 5-kinase, type I, gamma (PIP5K $\gamma$ ), an enzyme that phosphorylates phosphatidylinositol 4-phosphate (PIP2) to generate phosphatidylinositol-4,5-bisphosphate (PIP2). The mutation abolishes the kinase activity of PIP5K1C. Based on this finding, we sequenced genes in the LCCS2 locus that encode proteins in pathways interacting with the phosphatidylinositol pathway. We demonstrate that LCCS2 is caused by a mutation in *ERBB3* (*Her3*) which is known to modulate PI3K, an enzyme that phosphorylates PIP2 to produce phosphatidylinositol-3,4,5-triphosphate (PIP3). Thus, a defect in the phosphatidylinositol pathway leading to decrease in synthesis of PIP2, a molecule active in endocytosis of synaptic vesicle proteins, culminates in lethal congenital arthrogryposis.

#### C03.5

##### Drastic reduction in the life span of cystatin C L68Q gene carriers due to life-style changes in the last two centuries

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Hereditary cystatin C amyloid angiopathy (HCCAA) is an autosomal dominant disease with high penetrance, manifest by brain hemorrhages in young normotensive adults. In Iceland, this condition is caused by the L68Q mutation in the cystatin C gene, leading to amyloid deposition in cerebral arterioles, ending in the death of contemporary carriers at an average age of only 30 years. Here, we report, based both on linkage disequilibrium and genealogical evidence, that all known copies of this mutation derive from a common ancestor born roughly 18 generations ago. Intriguingly, the genealogies reveal that obligate L68Q carriers, in all families, born 1825 to 1900 experienced a drastic reduction in life span, from 65 years to the present day average. At the same time, a parent-of-origin effect emerged, whereby maternal inheritance of the mutation was associated with a 9 year reduction in life span relative to paternal inheritance. As these trends can be observed in several different extended families, three centuries after the mutational event, it seems likely that some environmental factor is responsible, perhaps linked to radical changes in the life-style of Icelanders during this period. A mutation with such radically different phenotypic effects in reaction to normal variation in human life-style not only opens the possibility of preventive strategies for HCCAA, it may also provide novel insights into the complex relationship between genotype and environment in human disease.

#### C03.6

##### Greater than 1% of Contemporary West Africans are Carriers of a Founder Mutation for Severe Recessive Type VIII OI, Which Was Presumably Brought to America with the Colonial Slave Trade and Also Occurs in African-Americans

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Classical Osteogenesis imperfecta (OI) is caused by a wide variety of dominant mutations, many de novo, in type I collagen and has an incidence of 1/20,000 births. Two recessive OI types, caused by defects in cartilage-associated protein (CRTAP) or prolyl 3-hydroxylase 1 (LEPRE1), account for approximately 5% of OI cases in North America. We identified a recurring mutation in LEPRE1, IVS5+1G>T (Nat Genet (2007) 39:359-365) in 6 probands (9/12 alleles) with severe/recessive type VIII OI (OMIM #610915). All probands had carrier

parents who were African-Americans or contemporary West-African immigrants to USA, suggesting the existence of a mutant allele which had been transported to America with the colonial slave trade. To estimate the carrier frequency of IVS5+1G>T in African-Americans, we extracted genomic DNA from 1429 random African-American newborn metabolic screening cards from Pennsylvania. We identified 1/286 carriers (0.35%), predicting a possible incidence of lethal type VIII OI due to IVS5+1G>T homozygosity of 1/330,000 African-American births. Furthermore, genomic DNA from contemporary West Africans was screened by SNP assay, followed by PCR confirmation of positive samples. Fifteen of 1097 independent individuals (1.37%) from Nigeria and Ghana were heterozygous for IVS5+1G>T. This high carrier frequency suggests that the incidence of type VIII OI in West Africa from this mutation alone is equivalent to the incidence of dominant OI. Haplotype data on probands, carriers and unaffected sibs supports the occurrence of a common founder mutation over 300 years ago, consistent with our hypothesis on the transportation of this West African allele to the Americas.

#### C04.1

##### Copy number variations in patients with overgrowth syndromes detected by array-CGH

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Overgrowth syndromes are a heterogeneous group of conditions including endocrine hormone disorders, several genetic syndromes and many situations with thus far unexplained mechanisms. Interestingly, chromosomal deletions and duplications have been identified in patients with overgrowth such as dup(4)(p16.3), dup(15)(q26-qter) and del(9)(q22.32-q22.33). Thus, we hypothesized that the sensitivity of array-CGH could improve the genetic diagnosis of overgrowth conditions.

Sixty-five patients with unexplained overgrowth syndrome were analyzed using a 1 Mb resolution array-CGH. The patients were classified into two groups: group I (32 cases) includes patients with a clinically known syndrome (i.e Sotos syndrome or Simpson-Golabi-Behmel syndrome) whereas group II (32 cases) includes patients with unclassified overgrowth syndrome.

We detected eight possibly pathogenic imbalances (12.3%) among 2 patients belonging to group I and 6 patients belonging to group II. Two are deletions and 6 are duplications. No recurrent abnormality was identified. FISH analyses confirmed the chromosomal abnormalities in 5 cases while the remaining cases are still under investigation.

Firstly, these results demonstrate that array-CGH is able to provide a high diagnostic yield in patients with overgrowth syndrome. Secondly, while chromosomal deletions are most often associated with growth retardation, we found that the majority of the imbalances detected in our patients are duplications. Thirdly, careful re-examination of patients may allow the delineation of novel clinically recognizable overgrowth syndromes. Finally, besides their importance for diagnosis and genetic counseling, these data may pave the way to the search of genes involved in the pathogenesis of overgrowth.

#### C04.2

##### Design, implementation and results using diagnostic oligo array-cgh

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Array-cgh is an essential tool for the detection, quantification and interpretation of cryptic copy number changes throughout the genome. Using Agilent Technology's eARRAY we have designed and customised a 60mer oligo array printed in 4x44K format (i.e. four 44,000 oligo arrays per slide). This constitutional array provides maximum even coverage of the genome and targets known microdeletion/duplication syndrome regions, is semi-automatable and provides a high-throughput platform for array-cgh. To date we have reported 350 diagnostic oligo arrays

(a) 85% ascertained following normal karyotypes but with idiopathic mental retardation, dysmorphism and/or congenital abnormalities; (b) 15% for further characterisation of visible chromosome abnormalities including apparently balanced translocations (ABCR), complex rearrangements (CCR) and supernumerary marker chromosomes (SMC). We have detected copy number changes (ranging in size from 88 Kb - 5 Mb) in 25% of ascertainment group (a), including a number of previously unreported de novo abnormalities. The majority of group (b) cases were found to have deletions or duplications not detected by light microscopy (ABCR and CCR), or arrays resolved their chromosomal origin and composition (SMC). Interpretation of array-cgh results remains challenging and the increasing use and resolution of this technology suggests that it may soon replace karyotyping for some primary ascertainment groups.

#### C04.3

#### The challenge of interpreting microduplications detected by arrayCGH

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The decision whether arrayCGH copy number alterations are causative for the phenotype comprises a number of steps: exclusion of known polymorphisms, confirmation by MLPA/FISH, analyzing parental samples, and a literature/database search to compare the phenotype with cases with similar genotypes. The interpretation of microduplications is difficult. FISH confirmation is technically not always possible and specific MLPA primers have to be constructed. The phenotype is usually variable and thus inheritance from a (near) normal parent does not always exclude a causal relationship. The scarcity of published cases is also a well known problem.

We analysed 300 karyotypically normal MR/MCA patients with an in house 6500k BACarray. We detected, besides polymorphic CNV's, 38 microduplications (0.3-7.3Mb) in 35 patients. In 28 cases FISH resulted in 18 confirmed (1.4-7.3Mb) and ten non-confirmed (0.3-3.4Mb) duplications. In eight of the ten non-confirmed cases the duplication was shown to be inherited by arrayCGH, demonstrating that FISH is not always suitable for confirming duplications. Three FISH non-confirmed duplications were tested and confirmed by MLPA. For 26 duplications the parents were tested: 19 were inherited (0.5-4.14Mb) and seven were *de novo* (0.3-7.3Mb). Upon re-evaluation one mother appeared to have a similar phenotype as her affected son. Of the 38 microduplications six still need confirmation, seven need parental testing, 18 are inherited from a phenotypically normal parent and three could not be confirmed. So far four seem to be clinically relevant; the *de novo* duplications 9p24.3, 17p13.3, and 19q13.31q13.32, and a maternally inherited duplication 22q13.3. Clinical information will be presented.

#### C04.4

#### Array-CGH analysis of MCA/MR patients: identification of 5 novel microdeletion syndromes

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We have investigated 84 patients with mild to severe mental retardation associated to facial dysmorphisms and/or congenital anomalies. All patients had a normal karyotype and have been evaluated by clinical geneticists (AR and FM) who excluded a recognizable syndrome on a clinical ground. Using 105K oligo Array-CGH a mean of 5 CNVs/patient were identified. These ranged in size from 62Kb to 1Mb and they are reported in the databases as benign polymorphisms. Private imbalances were detected in 24 out of 84 patients. In 10 cases (12%) a private rearrangement was inherited from one healthy parent. In 14 cases (16.5%) the rearrangement was *de novo*: 5 were novel deletions (Tab.1, cases 1-5) and 9 were known syndromes in atypical cases (Tab.1, cases 6-14). The last group included three cases of 22q11 deletions, the shortest 4p- known in the literature, and one case of Potocki-Lupski. The five novel *de novo* deletions ranged between 2.6 and 13.9Mb and they overlapped with polymorphic regions for an extent of 5-48%. Only two (6q24.3-q25.1 and 7q36.1-q36.2) are flanked by LCRs. An accurate search of the literature allowed to identify additional patients with overlapping deletions. Comparative analysis of the

phenotype of these patients with our patients suggested that a specific phenotype of these syndrome may be defined. These characteristics should be taken into account in order to identify additional patients.

Tab.1 *de novo* rearrangements

Case	Molecular karyotype	Presence of LCR	Overlap with polym. region	Number of genes	Disease genes	References
1	del(2)(q24.3q31.1)[10.6 Mb]	NO	5%	50	SCNA2; GALNT13; SCN1A; SCN9A; ABCB11; LRP2; BBS5; GAD1; ITGA6; CHRNA1	Pescucci et al.; Eur J Med Genet. 2007;50(1):21-32
2	del(2)(q31.2q32.3)[13.9 Mb]	NO	33%	43	CERKL; NEUROD1; FRZB; COL3A1; COL5A2; SLC40A1; PMS1; HIBCH; STAT1	Mencarelli et al.; Am J Med Genet A. 2007;43(8):858-65
3	del(6)(q24.3q25.1)[2.6 Mb]	SI	10%	21	SUMO4	Caselli et al.; Eur J Med Genet. 2007;50(4):315-21
4	del(7)(q36.1q36.2)[5.5 Mb]	SI	35%	56	CNTNAP2; KCNH2; NOS3; PRKAC2	Caselli et al.; Am J Med Genet A. 2007
5	del(14)(q12q12)[3.0 Mb]	NO	48%	5	COCH	
6	del(1)(p36.32p36.33)[3.8 Mb]	NO	70%	65	SKI; PEX1; TP73	
7	del(4)(p16.3p16.3)[2.0 Mb]	SI	60%	30	PDE6B; IDUA; FGFR3; WHSC1	
8	del(15)(q11.2q13.1)[5.7 Mb]	SI	35%	14	NDN; SNRPB;UBE3A; GABRB3; OC2A	
9	dup(17)(p11.2p11.2)[3.9 Mb]	SI	63%	41	TNFRSF13B; FLCN; RAI1; ATPAF2; MYO15A; ALDH3A2; AKAP10	Greco et al.; Clin Genet. 2008;73(3):294-6
10	del(17)(p11.2p11.2)[3.4 Mb]	SI	63%	41	TNFRSF13B; FLCN; RAI1; ATPAF2; MYO15A; ALDH3A2; AKAP10	
11	del(22)(q11.2q11.21)[3.1 Mb]	SI	42%	37	PROH; TBX1; COMT; RTN4R; SERPIND1; SNAP29; GGT2	Uliana et al.; Clin Dysmorphol. 2008;17(1):13-7
12						
13						
14	del(22)(q13.31q13.33)[4.7 Mb]	NO	54%	39	PPARA; TRMU; ALG12; MLC1; SC02; ECGF1; ARSA; SHANK3; ACR	

#### C04.5

#### Towards an improved genetic diagnosis of individuals with a congenital heart defects

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Detection of submicroscopic chromosomal imbalances by array-CGH opens opportunities in diagnostics as well as in the identification of novel loci involved in the patients phenotype. We analysed 130 patients with idiopathic 'syndromic' congenital heart defects (CHDs) by array-CGH with a 1Mb resolution. Causal chromosomal abnormalities were detected in 22/130 patients (17%).

In some of the regions, genes known to cause CHDs upon mutation are found: TBX1, NKX2.5, GATA4, NSD1, EHMT1, NOTCH1, ATRX and CBP. In the remaining regions no genes are known to cause CHDs, and they thus represent novel loci linked to CHDs. To identify the causal genes, the genes in imbalanced regions were first scored for their potential involvement in cardiogenesis using a tailored modular prioritisation algorithm based on ENDEAVOUR (Aerts et al, Nat Biotech 24 p 537). We first added expression microarray data of murine cardiogenesis to the ENDEAVOUR framework. We next constructed 6 different training sets related to different aspects of CHDs or cardiogenesis. Leave-one-out cross-validations determined which data sources contain relevant information for prioritization of genes related to that process. Next we fused these different prioritizations (of one candidate gene set, using different training sets) into one overall prioritization. We validated the prioritisation of ENDEAVOUR by expression analysis in zebrafish. 45 of the highest-ranking genes were analysed. As a positive control we analysed expression of some of the identified genes known to cause CHDs. Two strong candidate genes for CHDs emerge from these analyses: BMP4 and HAND2.

#### C04.6

#### Information management for constitutional cytogenetics: tools for ArrayCGH in a clinical diagnostic context

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As Microarray-CGH is introduced into clinical practice for identification of submicroscopic genomic aberrations, tools to handle related data become essential for clinical geneticists and biomedical researchers alike. We have developed Bench, a web application that combines a constitutional cytogenetics database and tools for search, visualisation, genome annotation, automated genotype-phenotype linkage,

reporting, and literature mining.

Array-CGH technology is currently on its way to replace classical karyotyping as primary diagnostic tool in copy number screening. Its vast importance in clinical diagnosis and research are underlined by large number of genes in human development and disease still unknown. A data storage and mining tool aimed specifically at leveraging the power of Array-CGH in a clinical context will aid aetiology of rare and complex genetic diseases by characterising genomic rearrangements, annotating and analysing clinical features, and providing advanced data mining and integration. For example, through an automated analysis of PUBMED abstracts, Bench allows to prioritize candidate genes in genomic deletions and duplications by phenotype characteristics annotated to the patient. Also, known Copy Number Variations, genes and their functions, and other genome annotations, when integrated with patient related data, aid in diagnosis and aetiology of new submicroscopic chromosomal imbalance syndromes. Bench provides a collaborative Array-CGH LIMS system that allows to maintain records of phenotype and chromosome rearrangements in patients, augmented with data mining, reporting and visualisation facilitating research, diagnostics, research and genetic counseling by involving relevant information from a variety of sources. Bench can be used free of charge in a research collaboration. <http://www.esat.kuleuven.be/cghgate/>.

#### C05.1

#### Functional interactions of conserved non-coding (CNC) sequences with other CNC using circular chromosome conformation capture (4C)

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The comparison of human chromosome 21 (Hsa21) sequences with the mouse syntenic regions led to the identification of roughly 3500 regions displaying an identity of >70% over a length of at least 100 nucleotides of ungapped alignment. About 65% (~ 2300) of these are conserved non-coding sequences (CNCs). Very little is known about the function of most CNCs. We speculated that a functional CNC may interact with its genomic target (i.e. an enhancer would bind to its cognate gene promoter). Thus, the identification of any part of the genome that interacts directly with a CNC could provide clues on the function of the latter. We have generated libraries of CNC-interacting DpnII fragments by chromosome conformation capture (4C) whose identity is determined by subsequent high-throughput sequencing.

We are currently screening for the interactions of 18 CNCs located in the two ENCODE regions of HSA21 in different cell lines. Preliminary results for two CNCs in K562 cells indicate that these may interact near regions of the genome that show conservation among vertebrates. Indeed, the median distances from the sequenced DpnII fragments to the nearest conserved region are 381.5 bp ( $P = 0.0583$ ) and 764bp ( $P = 0.023$ ) respectively for the 2 CNCs analysed. These results provide initial evidence that the function of CNCs is mediated by their interactions with other conserved regions. Interestingly, these CNCs are capable of interactions with loci not only in cis and over several Mb, but also in trans with loci located on other chromosomes.

#### C05.2

#### A high-resolution structural variation map of a human genome by next-generation, high-throughput paired-end sequencing

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The human genome and HapMap projects have considerably increased our understanding of the role of sequence variation in evolution and disease. Hybridization microarrays and fosmid-end sequencing reveal that structural variants (SVs) including insertions, deletions, duplications, inversions and translocations are common and extensive. Microarray methods, however, lack resolution and are blind to unbalanced events, while clone-based end-sequencing is time consuming and expensive. Here, we present a high-resolution survey of SVs of a

human genome, a HapMap Yoruba sample (NA18507), by ultra-high throughput sequencing of paired-end libraries with the AB SOLiD(TM) System. We sequenced a variety of 2x25-bp paired-end libraries (>15Gb) with insert sizes ranging from 600bp to 6kb (SD 10-23%). Each library provides over 10x physical (clone) coverage, with a total combined physical coverage >60x for 90% of the genome. The high physical coverage and diverse insert sizes allowed detecting small indels within tags (1-10 bp), and approximately 70,000 indels of length 20 bp to >100 kb. Additionally, we sequenced 7Gb of 50-bp fragment libraries, which combined with the paired libraries provided over 12x sequence coverage, allowing us to discover millions of SNPs of which 75% are found in dbSNP. Inferred SVs were compared to a database of end-sequence pairs of 10x physical coverage obtained by di-deoxy sequencing of 40kb fosmid ends. A subset of novel SVs were validated by PCR and Sanger sequencing. Our results serve as a model for further high-resolution exploration of genetic variation in human populations and cancer with next-generation sequencing.

#### C05.3

#### Expression analysis using deep Solexa sequencing shows major advances in robustness, resolution and inter-lab portability over microarray platforms

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The hippocampal transcriptomes of wild-type mice and mice transgenic for δC-doublecortin-like kinase were analyzed with the Solexa deep sequencing technology. We determined around 2 million sequence tags per sample and compared these data with results of the same samples analysed using five different microarray platforms. Seventy percent of the sequence tags were mapped to approximately 30,000 unique, high-confidence transcripts, their abundance spanning four orders of magnitude. Antisense transcription, undetectable by microarrays, was found in 51% of all genes, and alternative polyadenylation in 47%. With a dedicated Bayesian model and false discovery rate of 8.5%, we measured statistically significant differential expression for 3179 transcripts; many more and with higher fold-changes than observed using microarrays. The deep sequencing technology demonstrates superb reproducibility, not only between biological replicates but even across laboratories. The described major advance in robustness, comparability and richness of sequence -based transcriptomics data is expected to boost in-depth collaborative, comparative and integrative genomics studies.

#### C05.4

#### Studying gene dosage imbalance in embryonic stem cells

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To gain insight into the alterations of the transcriptional pathways underlying the pathogenesis of Down syndrome, we decided to use an integrated strategy combining the systematic overexpression of chromosome 21 genes in ES cells, transcriptome analysis and systems biology approaches.

We generated an ES cell clone bearing an inducible/exchangeable cassette in the ROSA26 locus to be used to insert, one by one, every murine orthologs of human chromosome 21 genes. Using this flexible system, we developed a library of ES cells over-expressing, in an inducible manner, murine orthologs of human transcription factors, kinases and miRNAs mapping on HSA21 in order to perturb the physiological genetic network at the cellular level. The biological pathways affected by the over-expression of each coding and non-coding gene and their regulators and regulated genes have been inferred using system biology approach. We already mapped the regulatory gene networks specifically altered by each transcription factors and kinases, opening new hypothesis toward the understanding of pathogenesis of Down syndrome.

Finally, the above-described ES cell lines have been used to study the gene dosage imbalance effects on ES cell differentiation to cardiomyocytes, myeloid and neuronal lineages, tissues affected by the DS. This project represent the first to involve a systematic overexpression

of all individual genes from a single human chromosome (HSA21) in mouse ES cells to identify the effects of gene dosage imbalance on the global transcriptome and on the ability of pluripotent ES cells to differentiate into lineages relevant to human aneuploidy phenotypes.

#### C05.5

#### A large human miRNA library screen reveals a potential role of miRNAs in the fine tuning of fibrinogen levels

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In humans, plasma fibrinogen levels are maintained between 2-4 g/L with a wide variability in the population. Here, we addressed the role of microRNAs in the regulation of fibrinogen gene expression by screening the regulatory potential of 471 human microRNAs (from the ~1'000 annotated miRNAs in the human genome) on all five fibrinogen transcript 3'UTRs (FGA, FGAA-E, FGB, FGG and FGG'). For this purpose, we cloned each fibrinogen 3'UTR behind a firefly luciferase reporter gene. Co-transfections in HEK-293 cells included one of the firefly luciferase reporter gene constructs, a microRNA precursor and a transfection efficiency control (expressing renilla luciferase). The regulatory effects of microRNAs on each fibrinogen 3'UTR was calculated by dividing the ratio of luciferase intensities (firefly/renilla) of each transfection with a microRNA by the ratio from a reaction without microRNA. We also screened human liver RNA for expression of 362 microRNAs, by qPCR. 57% of the tested microRNAs are expressed in the liver. With these experiments, we identified 8 microRNAs, expressed in the liver, showing -13% to -40% down-regulating potential; 4 of these acting specifically on FGA, 2 on FGB, 1 acting on FGA and FGG and 1 down-regulating all fibrinogen 3'UTRs. None of these microRNAs showed an effect on the control firefly luciferase vector lacking a 3'UTR, demonstrating fibrinogen 3'UTR-specific down-regulation potential. We also detected 16 liver-expressed microRNAs with up-regulating potential of + 16% to + 176%. Ongoing experiments are assessing the effects of these candidate microRNAs on endogenous fibrinogen synthesis in HepG2 cells.

#### C05.6

#### Visualization of molecular interactions *in situ*, with single-molecule resolution

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New, powerful techniques are required to observe individual protein molecules and their interactions *in situ*, in order to analyze, with high precision, in which cells the interacting proteins are located, and in what sub-cellular compartments.

We therefore developed a method called *in situ* proximity-ligation assay (*in situ* PLA) that requires multiple recognition events, by a pair of antibodies, for detection. By conjugating DNA-strands to the antibodies we convert the binding of the antibodies to a protein or a protein complex into an amplifiable DNA molecule, thus increasing both the selectivity and sensitivity of the assay. Rolling-circle amplification generates a concatemeric product for localized detection by fluorescently labeled probes. Using the *in situ* PLA, we could detect interactions between two (c-Myc/Max) and three (c-Myc/Max/RNA pol II) endogenous proteins, visualizing the active fraction of c-Myc as it exert its function in promoting gene transcription (Söderberg *et al.* *Nature Methods*, 2006), as well as post-translational modifications, i.e. phosphorylation of PDGFRβ, in cultured cells and fresh frozen tissues sections *in situ* (Jarvius *et al.* *Molecular & Cellular Proteomics*, 2007). *In situ* PLA is applicable for detection of all types of biomolecules (e.g. proteins, DNA and RNA) and interactions thereof, allowing detection and enumeration of biomolecules within cells and tissue, at a single-molecule resolution. As the read-out of the method is based upon DNA amplifications, *in situ* PLA can easily be multiplexed for simultaneously detection of multiple analytes. *In situ* PLA provides a unique opportunity to monitor interaction patterns for diagnostic purposes.

#### C06.1

#### Mutations in the Cyclin family member FAM58A cause a novel X-linked dominant disorder characterized by syndactyly, telecanthus, anogenital and renal malformations (STAR syndrome)

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We recently identified four girls with a consistent constellation of toe Syndactyly, Telecanthus, Anogenital and Renal malformations, and we propose the name STAR syndrome for this condition. A single mother-daughter pair had previously been reported by Green *et al.* with a similar combination of malformations. The authors noted that this condition was autosomal dominantly inherited and overlapping with but distinct from Townes-Brocks syndrome (OMIM #601446). Array CGH performed with DNA of one of our patients revealed a de novo heterozygous deletion of 37.9-50.7 kb including exons 1 and 2 of the gene FAM58A on Xq28, and qPCR detected a de novo deletion of FAM58A exon 5 in a second case. Point mutation analysis revealed one truncating and two splice mutations in FAM58A in three further cases including the family reported by Green thus confirming this disorder as a distinct recognizable X-linked dominant condition. FAM58A encodes a Cyclin box fold domain, and in accordance with that siRNA mediated knockdown in cultured cells revealed a proliferation defect. FAM58A interacts with SALL1 but not SALL4 as determined by co-immunoprecipitation, corresponding to the close phenotypic overlap with Townes-Brocks syndrome.

#### C06.2

#### KCNQ2 mutations and implications for counselling and perinatal care in Benign Familial Neonatal Convulsions

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**Background:** Benign familial neonatal convulsions (BFNC) is an autosomal dominantly inherited form of epilepsy characterized by seizure onset around the third day of life, spontaneously resolving within a few months. In general, psychomotor development is normal, but 10-15% of patients develop epilepsy later in life. BFNC is caused by mutations in the voltage-gated potassium channel subunit gene KCNQ2 (20q13.3) or, less frequently, KCNQ3 (8q24). Purpose of this study is to determine the frequency of KCNQ2-mutations in BFNC families and sporadic cases.

**Methods:** MLPA- and sequence analysis of the KCNQ2 gene was performed in 28 probands/families with neonatal seizures. Larger deletions were analysed with Infinium humanhap300 SNP-array. Phenotypic details were provided by the referring physician.

**Results:** In eleven families and three sporadic cases, three missense, eight frameshift or nonsense mutations, and three large deletions were detected. All large deletions (ranging from 49 to 479 kbp) contained one to fourteen annotated genes, including the frontal lobe epilepsy gene, CHRNA4, in two families. Associated mental retardation (without active epilepsy), autism or (febrile) seizures later in life, occurred in multiple families. Perinatal mutation analysis was performed in two families.

**Conclusions:** Mutations were found in 50% of probands with (benign) neonatal convulsions. Large deletions comprise a substantial portion (21%) of KCNQ2-mutations. Mutation detection in neonates with benign convulsions can prevent superfluous diagnostic procedures. Furthermore, perinatal mutation analysis in sibs with 50% risk to BFNC could significantly improve perinatal management.

**C06.3**

**Clinical features of maternal uniparental disomy 14 are also present in patients with an epimutation and a deletion of the imprinted DLK/GTL2 gene cluster**

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<sup>8</sup>Institut für Humangenetik, Christian Albrecht Universität Kiel, Kiel, Germany. Maternal uniparental disomy 14 [upd(14)mat] is associated with a recognizable phenotype that includes pre- and postnatal growth retardation, neonatal hypotonia, feeding problems and precocious puberty. Chromosome 14 contains an imprinted gene cluster, which is regulated by a differentially methylated region (IG-DMR) between *DLK1* and *GTL2*. Here we report on six patients with clinical features of maternal upd(14)mat who show a typical methylation pattern at the IG-DMR and the *GTL2* promoter region, but biparental inheritance for chromosome 14. In five of the patients loss of paternal methylation appears to be a primary epimutation, whereas the other patient has a paternally derived deletion of ~1 Mb that includes the imprinted *DLK-GTL2* gene cluster. These findings demonstrate that the upd(14)mat phenotype is caused by altered expression of genes within this cluster.

**C06.4**

**Capillary Malformation - Arteriovenous Malformation: clinical and molecular aspects**

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Background: Mutations in *RASA1* were documented in 6 families (39 individuals) with autosomal dominant multifocal capillary malformations (CMs). Nine individuals had an associated arteriovenous malformation/fistula (AVM/AVF). One patient had Parkes Weber syndrome (PKWS), a disorder considered to be sporadic and non-genetic.

Methods: We collected clinical information and DNA samples for 61 probands (from 21 centers) and their families with a phenotype similar to that observed in the original study: 56 had multifocal CMs, and 35 also a fast-flow vascular anomaly: 19 AVM/AVF and 16 PKWS; 5 had PKWS without multifocal CMs. *RASA1* was screened by DHPLC followed by sequencing.

Results: We identified 42 distinct mutations in 44/61 probands: 16/19 with AVM/AVF, 13/16 with PKWS, 15/21 with multifocal CMs only, and 0/5 with PKWS without multifocal CMs. *RASA1* mutation was also found in 57 relatives. Overall, 17 individuals with a *RASA1* mutation had an AVM/AVF: 8 were intracranial, 2 of which were vein of Galen aneurysmal malformations. Moreover, 7 patients had either a benign or a malignant tumor, 3 of which are known to occur in neurofibromatosis type 1 or 2. Penetrance of *RASA1* mutations was 98% and de novo occurrence was 32%.

Conclusions: Multifocal CM is the hallmark of *RASA1* mutation. These patients often have extra- or intracranial AVM/AVF. This study confirms the original association designated capillary malformation-arteriovenous malformation (CM-AVM). In addition, PKWS, as well as vein of Galen aneurysmal malformation are genetic diseases, part of the CM-AVM spectrum. Specific neural tumors also may be linked to *RASA1*.

**C06.5**

**A novel, autosomal dominant, Pseudoxanthoma Elasticum-like phenotype in a five-generation family**

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive (AR) progressive disorder of elastic fibres characterised by dermal, ocular and vascular lesions, due to mutations in *ABCC6*, an ATP-binding cassette transporter, in ~80% of cases. Autosomal dominant (AD) inheritance is very rare and usually due to pseudodominance (Plomp et al. 2004). We studied 17 individuals from a 5-generation family with PXE-like skin manifestations. Many subjects also suffer premature claudication pain and ischaemic heart disease but they do not have typical PXE ophthalmic signs. Electron microscopy analysis of skin was normal except in one patient who had fragmentation and clumping of elastic fibres with no evidence of calcification. Sequencing of *ABCC6* was negative and linkage to this locus excluded. Vanakker et al (2007) reported *GGCX* mutations in 3 families with an AR PXE-like phenotype and vitamin K-dependent coagulopathy. Our family has no history of abnormal bleeding, clotting assays were normal in one affected individual, and sequencing of *GGCX* and *VKORC1* was normal. Immunohistochemistry of lesional skin tissue showed disturbance in the gamma-carboxylation of vitamin K-dependent mineralization inhibitors, particularly matrix gla protein (MGP). This suggests involvement of a related pathway as in the PXE-like phenotype of Vanakker et al (2007). Genome-wide linkage results, and candidate gene sequencing, are awaited. This 5-generation family with a PXE-like phenotype demonstrates unequivocal AD inheritance. We believe this is a previously unreported clinical and genetic entity, the pathogenesis of which may provide new information on the molecular pathways involved in PXE and related disorders.

**C06.6**

**Clinical phenotypes and outcome of 101 LMNA gene mutation carriers**

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Background. Lamin A/C (LMNA) gene mutations cause a variety of phenotypes. In the cardiologic setting, patients diagnosed with idiopathic dilated cardiomyopathy (DCM) plus atrio-ventricular block (AVB) constitute the majority of reported cases.

Methods. This was a longitudinal retrospective study conducted in 32 consecutive families in which LMNA gene defects were identified in the probands, sharing DCM (n=31) and ARVD (n=1) phenotype.

Results. Of the 171 family members, 101 were carriers of LMNA gene mutations. 65 of 101 (64.5%) were phenotypically affected while 36 were only genotypically affected, including 5 with preclinical signs. The 65 patients had DCM with AVB (n=44), DCM with Ventricular Tachycardia/Fibrillation (VT/VF) (n=12), DCM with AVB and Emery-Dreifuss Muscle Dystrophy type 2 (EDMD2) (n=6), AVB plus EDMD2 (n=2) and ARVD (n=1). The disease was proven to be familial autosomal dominant (AD) in 23 of the 32 families; likely familial AD in 6 and associated with a de novo mutation in 3. During a median follow-up of 57.4 months (interquartile range 26-115 months) we observed 54 events in 47 DCM patients (7 had a later event excluded from the analysis) whereas no event was observed among the 36 non-affected carriers. The events were related to heart failur

Conclusions. DCMs caused by LMNA gene defects are highly penetrant, adult-onset, malignant diseases characterized by high rate of HF and life-threatening arrhythmias predicted by NYHA class, competitive sport activity and type of mutation. We also found a LMNA gene defect associated with an ARVD clinical phenotype.

**C07.1****Oligosaccharyltransferase subunits mutations in non-syndromic mental retardation**

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Mental Retardation (MR) is the most frequent handicap among children and young adults. While a large proportion of X-linked MR genes have been identified, only four genes of autosomal recessive non-syndromic MR (AR-NSMR) have been described so far.

Here, we report on two new genes involved in autosomal and X-linked NSMR. First, autozygosity mapping in two sibs born to first cousin French family led to the identification of a region on 8p23.1-p22. This interval encompasses the gene *N33/TUSC3* encoding one subunit of the oligosaccharyltransferase (OTase) complex which catalyses the transfer of an oligosaccharide chain on nascent proteins, the key step of N-Glycosylation. Sequencing *N33/TUSC3* identified a one base-pair insertion, c.787\_788insC, resulting in a premature stop codon, p.N263fsX300, and leading to mRNA decay. Surprisingly, glycosylation analyses of patient fibroblasts showed normal N-glycan synthesis and transfer. Subsequently, screening the X-linked *N33/TUSC3* paralog, the *IAP* gene, identified a missense mutation (c.932T>G, p.V311G) in two brothers with X-linked NSMR. Interestingly, quantitative RT-PCR analyses showed increased *IAP* expression in *N33/TUSC3* mutated cells, suggesting that normal N-glycosylation observed in patient fibroblasts may be due to functional compensation.

Recent studies of fucosylation and polysialic acid modification of neuronal cell adhesion glycoproteins have shown the critical role of glycosylation in synaptic plasticity. However, our data provide the first demonstration that a defect in N-Glycosylation can result in NSMR. Altogether, our results demonstrate that fine regulation of OTase activity is essential for normal cognitive function development, providing therefore new insights to understand the pathophysiological bases of MR.

**C07.2****Influence of Friedreich ataxia GAA non-coding repeats expansions on pre-mRNA processing**

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The intronic GAA repeat expansion in the frataxin gene causes the hereditary neurodegenerative disorder Friedreich's ataxia. While it is generally believed that GAA repeats block transcription elongation, a direct proof in eukaryotic system is lacking. We tested in hybrid minigenes the effect of GAA and TTC repeats on nascent transcription and pre mRNA processing. Unexpectedly, disease-causing GAA repeats (n=100) did not affect transcriptional elongation in nuclear HeLa RUN ON assay nor pre mRNA transcript abundance but resulted in a complex defect on pre mRNA processing. GAA but not TTC repeats insertion downstream of reporter exons resulted in their partial or complete exclusion from the mature mRNAs and in the generation of a variety of aberrant splicing products. Interestingly, the GAA expansion induced the accumulation of an upstream pre mRNA splicing intermediate, which is not turned over into mature mRNA. This effect of GAA repeats was observed to be position and context-dependent, as their insertion at different distance from the reporter exons had a variable effect on splice site selection. Reduction of GAA triplets partially restored normal splicing consistent with a repeat length dependent phenotypic variability.

This data indicates, for the first time, an association between GAA non-coding repeats and aberrant pre-mRNA processing and suggests an alteration of the coordination between transcription and pre-mRNA processing in this disease. Transcribed GAA repeats might create a "decoy" exon that binding to trans acting splicing factors may interfere with normal turnover of non-coding intronic RNA leading to degradation and lower transcript levels.

**C07.3****Mechanisms of MeCP2 function underlying Rett syndrome as revealed from overexpression and knock-down systems in vitro**

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Rett syndrome is a severe X-linked neurodevelopmental disorder mainly affecting girls and is the second common cause of mental retardation in girls after the Down syndrome. The methyl CpG-binding protein 2 (MeCP2), a ubiquitous transcriptional repressor interacting with the chromatin remodeling machinery, is considered a single causative factor of Rett syndrome and related phenotypes, and some autistic cases. Our present study is focusing on interaction between MeCP2 and chromatin proteins leading to changes in chromatin architecture and silencing of gene expression. Using confocal microscopy, we demonstrate that MeCP2 protein is localized at the nuclear heterochromatin compartment together with the heterochromatin protein 1 alpha (HP1α). In addition, we have developed in-vitro systems overexpressing the normal MeCP2 and MeCP2 containing Rett related mutations, as well as a knock-down system of the endogenous MeCP2 using specific siRNA. Studying these systems with a comprehensive gene regulation antibody array, we demonstrate that the expression of a specific set of nuclear proteins, including hBRM/hSNF2a component of SWI/SNF, HMGB1 high mobility group protein, G9a histone methyltransferase, PRMT1 protein arginine methyltransferase and HDAC2 histone deacetylase, is synchronized with MeCP2 overexpression and knock-down. Moreover using co-immunoprecipitation analyses, we demonstrate a direct interaction between MeCP2 and hBRM/hSNF2a component of ATPase-dependent SWI/SNF complex involved in global chromatin remodeling mechanism. Our findings suggest that MeCP2 acts through parallel mechanism of chromatin remodeling involving HDACs and SWI/SNF complex, thereby inducing local as well as large scale changes in chromatin architecture and compaction.

**C07.4****Mutations in *UBE1* are associated with X-linked infantile spinal muscular atrophy (XL-SMA) and cause decreased gene expression in patients and carrier females**

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X-linked infantile spinal-muscular atrophy (XL-SMA; MIM301830) is a rare X-linked disorder that presents with the clinical features of hypotonia, areflexia and multiple congenital contractures (arthrogryposis) associated with anterior horn cell loss and infantile death. Large scale mutation analysis in the linkage interval (DXS8080-DXS7132) resulted in the detection of three rare novel variants in exon 15 of the gene coding for the Ubiquitin-Activating Enzyme E1 (*UBE1*): two missense mutations (c.1617 G>T, p.Met539Ile, c.1639 A>G, p.Ser547Gly) present each in one XL-SMA family and one synonymous C>T substitution (c.1731 C>T, p.Asn577Asn) identified in additional four unrelated families. Each of these variants was demonstrated to segregate with the disease. Absence of the missense mutations was demonstrated for 3550, absence of the silent mutation was shown in 7914 control X-chromosomes. These results yielded statistical significant evidence for the association of the silent substitution and the two missense mutations with XL-SMA ( $P = 2.416 \times 10^{-10}$ ,  $P = 0.001815$ ). We have also demonstrated that the silent C>T substitution leads to significant reduction of *UBE1*-expression in patients and interestingly to a lesser extent also in carrier females and alters the methylation pattern of exon 15, implying a plausible role of this DNA element in developmental *UBE1*-expression in humans. *UBE1* catalyzes in the ubiquitin-proteasome system (UPS) the first step in ubiquitin conjugation to mark cellular proteins for degradation. Our observations indicate that XL-SMA is part of a growing list of neurodegenerative disorders associated with defects in the ubiquitin-proteasome pathway.

**C07.5**

**VLDLR (very low density lipoprotein receptor) is the first gene implicated in cerebellar hypoplasia and quadrupedal locomotion in humans**

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Quadrupedal gait in humans is a rare phenotype. We studied four consanguineous families, reported as Unertan syndrome, all exhibiting quadrupedal gait, dysarthric speech, mental retardation, and varying degrees of cerebro-cerebellar hypoplasia. Homozygosity mapping linked the locus for this unique autosomal-recessive trait to chromosome 9p24 in families A & D and 17p13 in family B. Family C excluded linkage to both loci. These results suggest that hereditary disorders associated with quadrupedal gait are genetically heterogeneous. The 9p24 region includes the gene VLDLR, which is a component of the reelin signaling pathway. Sequence analysis of VLDLR revealed two distinct mutations, R257X in family A, and I780TfsX3 in family D. Both of these mutations presumably truncate the protein, apparently leaving behind a non-functional product. Unlike chromosome 9p24, the chromosome 17p interval is large and contains at least 157 genes. We adopted a bioinformatics approach to screen the 17p interval for trinucleotide repeats GAA, CAG, CGG and CTG, and neuronal expression. This analysis revealed several genes including lissencephaly-1 (LIS1, alternative symbol PAFAH1B1). LIS1 interacts with VLDLR, and heterozygous LIS1 mutations in humans cause lissencephaly I. Neither LIS1 nor four additional genes (WDR81, RUTBC1, MNT, TRPV1) showed an expansion of their repeat sequences. The search for the chromosome 17p13 gene in Family B continues. Our data suggest that mutations in VLDLR impair cerebro-cerebellar function and confer a dramatic influence on gait in humans.

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**C07.6**

**The utilization of T3/T4 screening of males with MR of unknown etiology to identify patients with Allan-Herndon-Dudley syndrome**

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Allan-Herndon-Dudley syndrome (AHD; OMIM 309600) is an X-linked recessive disorder presenting with hypotonia progressing to spasticity, delay in developmental milestones, and severe mental retardation. Mutations in the *MCT8/SLC16A2* gene have been shown to be causative in clinically diagnosed AHD patients. MCT8 functions as a thyroid hormone transporter with an essential function in the transport of triiodothyronine (T3) into neurons. An imbalance of certain plasma thyroid hormones has been identified in AHD patients. To investigate the clinical utility of thyroid screening in patients with MR, we measured free T3 and free T4 in a cohort of 137 males with MR of unknown etiology. Twenty males were identified to have elevated T3 and molecular analysis of *MCT8* in these patients identified two (10%) with pathogenic changes. One mutation was an insertion of 2 amino acids, p.G41\_S42 dup, in exon 1. The 40 year old male had profound MR, was ambulatory and non-verbal. He also had a seizure disorder and a right spastic hemiparetic arm. The second mutation (p.G282C) was in a 5 year old boy with truncal hypotonia and hypertonia of his extremities with spasticity, dystonia and hyperreflexia - all consistent with AHD. Additionally, 28 males enrolled in our XLMR study, with clinical or biochemical evaluations suggestive of AHD, were analyzed for *MCT8* mutations and seven (25%) had pathogenic changes. Based on our results, testing the T3 level may serve as a general screen for the AHD syndrome and should certainly be considered in males with significant mental retardation and hypotonia.

**C08.1**

**The role of the interferon regulatory factor 5 gene in autoimmune diseases**

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IRF5 is a transcription factor involved both in the type I interferon and toll-like receptor signalling pathways. We have found polymorphisms in the IRF5 gene to be associated with several autoimmune diseases; systemic lupus erythematosus<sup>1</sup>, rheumatoid arthritis<sup>2</sup>, multiple sclerosis<sup>3</sup> and inflammatory bowel disease<sup>4</sup>. By statistical methods two variants in the IRF5 gene have been shown to be independently associated to SLE. The variants are a CGGGG insertion-deletion (indel) polymorphism located in the promoter region of the IRF5 gene and a SNP rs10488631 located at the 3' end. The indel contains 3 or 4 repeats of the sequence CGGGG and the longer allele confers risk to all of the autoimmune diseases tested. The risk allele contains an additional binding site for the transcription factor SP1. Using electrophoretic mobility shift assays (EMSA) we observed allele-specific differences in protein binding to the indel and by proximity ligation assay (PLA) we demonstrated increased binding of the transcription factor SP1 to the risk allele.

Our study adds to the evidence that there might be genes or pathways that are common in multiple autoimmune diseases and that the type I interferon system is likely to be involved in the development of these diseases.

1) Sigurdsson S et al. Hum Mol Genet (2007) Dec 6

2) Sigurdsson S et al. Arthritis Rheum (2007) Jul;56(7):2202-10

4) Kristjansdottir G, Sandling J et al. J Med Genet accepted manuscript

3) Dideberg V et al. Hum Mol Genet (2007) Dec 15

**C08.2**

**Elevated expression of serotonin receptor type 3 genes may contribute to irritable bowel syndrome with diarrhea**

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Serotonin type 3 (5-HT<sub>3</sub>) receptor antagonists are beneficial in some but not all patients with irritable bowel syndrome and diarrhea (IBS-D). As cis-regulatory variants can play a role in the etiology of complex conditions by affecting efficiency of translation, we investigated the 5' and 3' untranslated region (UTR) of the 5-HT<sub>3A</sub> and 5-HT<sub>3E</sub> subunit genes. Mutation analysis was carried out in 200 patients with irritable bowel syndrome and 100 healthy controls. We found a *HTR3A* 5'UTR variant and a novel *HTR3E* 3'UTR variant associated with the IBS-D subtype. Functional studies showed that both variants lead to significant upregulation of subunit expression. In HEK293 cells, the *HTR3A* variant results in a higher density of 5-HT<sub>3A</sub> receptors at the cell surface compared to the wild-type control. The *HTR3E* variant affects a microRNA binding site and leads to a higher luciferase reporter gene expression. Both *HTR3E* and the miRNA co-localize in enterocytes of the mucosal cell layer of the gut epithelium as shown by *in situ* hybridization. We suggest that the increased expression of 5-HT<sub>3A</sub> and 5-HT<sub>3E</sub> subunits might result in a change in 5-HT<sub>3</sub> receptor composition and/or density of 5-HT<sub>3</sub> receptors in the epithelial cell layer of the mucosa and neurons of the enteric and central nervous system and could therefore contribute to the pathophysiology of IBS-D.

**C08.3****Genome-wide association scan for serum TSH levels in 2375 Sardinians**

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Thyroid stimulating hormone (TSH), the key regulator of thyroid function, regulates the growth of the thyroid gland and the secretion of the thyroid hormones T4 and T3. Thus, serum TSH concentrations are a sensitive indicator of thyroid function, with increased levels indicative of primary thyroid failure (hypothyroidism) and decreased level indicative of thyroid hormone overproduction (hyperthyroidism); however specific gene variants that influence levels are not known. To identify genetic factors associated with variation in TSH levels, we conducted a genome-wide association scan in 2375 Sardinians volunteers from 564 families participating to the ProgeNIA project, genotyped using the Affymetrix 500K and Affymetrix 10K Mapping Array. We evaluated the additive effect of 362,129 SNPs that passed quality controls, adjusting the model for familiality and covariates. To control inflation of type I error due to outliers and departure from normality, quantile normalization was applied prior the analysis. The GWA scan revealed several loci putatively associated with serum TSH level and thus we followed up all the markers reaching the genome-wide significance threshold of  $1.3 \times 10^{-7}$  in an internal independent Sardinian cohort of 1903 volunteers, 1164 Tuscans samples from the InCHIANTI study, and 987 Amish samples from the HAPI study. Replication is ongoing, but preliminary results support the finding for the top associated chromosome 5 SNP (overall pvalue  $<10^{-14}$ ), with the same allele responsible for an increase in TSH levels in all studies. Further detailed SNP analysis of the gene is necessary to define the causal variant and verify its direct implication on thyroid related pathologies.

**C08.4****BCL11A is associated with persistent HbF and ameliorates the β-thalassemia phenotype**

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β-thalassemia and sickle cell anemia are among the most common inherited disorders worldwide, with a marked phenotypic heterogeneity related to environmental and/or genetic factors. The reasons for this are not well understood, although the level of fetal hemoglobin (HbF) is one well characterized ameliorating factor in both of these conditions. To dissect the genetic basis of this heterogeneity we conducted genome wide scans with 362,129 common SNPs on 4,305 Sardinians to look for genetic linkage and association with fetal hemoglobin levels. Our approach allowed us to identify genetic variants at the BCL11A locus strongly associated with this trait ( $p < 10^{-35}$ ). Importantly, we have further shown that this BCL11A variant, by influencing the HbF levels, may moderate the phenotype of homozygous β-thalassemia. The fre-

quency of the C allele of SNP rs11886868, in the BCL11A gene, was significantly higher in Sardinian individuals with elevated HbF levels and patients with attenuated forms of β-thalassemia versus those with thalassemia major, detected by screening for β-thalassemia. We also showed that the same BCL11A variant is strongly associated with fetal hemoglobin levels in a large cohort of sickle cell patients.

These results indicate that BCL11A variants, by modulating HbF levels, act as an important ameliorating factor of the β-thalassemia phenotype, and it is likely they could help in moderating other hemoglobin disorders. Further genetic and functional studies are in progress to characterize the molecular mechanisms of fetal globin regulation, and could eventually lead to the development of new therapeutic approaches for β-thalassemia and sickle cell anemia.

**C08.5****Genome-wide association study and follow up: identification of novel coeliac disease determinants related to the immune response**

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Coeliac disease (CD) is a common inflammatory intestinal disease, caused by an immune response to wheat, rye and barley. HLA-DQ2/8 is necessary, but not sufficient for disease development.

To search for additional genetic factors predisposing to CD we have performed genome-wide associations (GWA) study in 778 coeliac cases and 1422 controls from the UK using the Illumina HumanHap300 BeadChip. Above the strong and extended association to the HLA region, the 4q27 genomic locus, including two immune related genes *IL2* and *IL21*, was associated at the genome-wide significance level ( $p=2.0 \times 10^{-7}$ ). Association with *IL2/IL21* gene locus was confirmed in three independent coeliac populations. Moreover, we observed similar association of *IL2/IL21* gene region in other autoimmune diseases (type 1 diabetes, rheumatoid arthritis), suggesting it as the common autoimmune locus. In a more extensive follow up we investigated 1,020 top genome-wide associated SNPs in multiple independent cohorts from three populations (5,049 samples). We identified seven new loci ( $p < 5.0 \times 10^{-7}$ ), six of which contain genes controlling adaptive immune responses, including: *CCR3*, *IL12A*, *IL18RAP*, *RGS1*, *SH2B3* and *TAGAP*. Three novel CD loci (*IL2/IL21*, *CCR3* and *SH2B3*) overlap with genomic regions that show association to type 1 diabetes (HLA-mediated autoimmune disorder), whereas association in the *IL18RAP* loci overlap with another intestinal inflammatory condition - Crohn's disease. In the GWA study and the follow up we have identified new biological mechanisms for coeliac disease. This study shows the power of GWA studies with proper replication to uncover the genetics of complex traits.

**C08.6****Genome-wide association and functional studies identify SLC2A9 (GLUT 9) as a novel uric acid transporter influencing serum urate concentration, urate excretion and gout disease**

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**Aim.** Uric acid is the end product of purine metabolism. Humans have lost hepatic uricase activity, leading to uniquely high serum uric acid concentrations compared with other mammals. We aimed to study genetic regulation of serum concentration of uric acid (SUA).

**Materials and methods.** A genome-wide association scan using

317,503 single nucleotide polymorphisms (SNPs) was carried out in a sample of 986 individuals from Vis Island, Croatia, in which about 100 quantitative traits were (QT) measured. Pedigree-based genome-wide QT locus association method was used.

**Results.** SUA was strongly associated with three SNPs ( $P \leq 10^{-7}$  after Bonferroni correction), implicating the role of the major facilitator superfamily member *SLC2A9* and explaining 1.7-5.3% of the variance in SUA concentrations. We then confirmed the association between *SLC2A9* variants, low fractional excretion of uric acid and gout disease in several other population samples from United Kingdom, Croatia and Germany. In all samples the effects were substantially greater in females. A meta-analysis showed odds ratio for gout between 1.34 and 1.38 for the three implicated SNPs: rs1014290 T allele, rs6449213 T allele and rs737267 C allele. As *SLC2A9* was a known fructose transporter, we conducted several functional experiments in *Xenopus* oocytes and showed strong uric acid transport activity of *SLC2A9*. The uptake of uric acid was 7-fold greater than of the known urate transporter *URAT1* and 31-fold than in the control oocytes.

**Conclusion.** *SLC2A9* (*GLUT 9*) is a novel uric acid transporter influencing serum urate concentration, urate excretion and gout disease.

#### C09.1

#### QF-PCR as a stand-alone prenatal test for targeted referral groups; results from the first year of a new UK diagnostic service

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QF-PCR is widely used in European genetic laboratories as a rapid, robust and accurate test for the prenatal diagnosis of trisomy of chromosomes 13, 18 and 21. To date, our laboratory has tested more than 25,000 samples using both QF-PCR and karyotype analysis. Recent audits have found a low incidence of clinically significant non-trisomy chromosome abnormalities in prenatal samples referred for increased trisomy risk, which has led to debate in the UK over the clinical need for karyotyping such samples. In May 2007, the SE England Genetics Consortium therefore instigated a QF-PCR stand-alone service 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 and 21 using QF-PCR. Karyotype analysis is carried out only for referrals with NT >3mm before 14 weeks or >6mm thereafter, ultrasound abnormalities (excluding single soft markers), from families with a previous chromosome abnormality (excluding common trisomies) and to follow up abnormal QF-PCR results. During the first eight months of service, 1502 AF and 628 CVS were received. 26% of AF and 44.9% of CVS were karyotyped. 8% of AF and 22% of CVS were abnormal. In the groups targeted for karyotyping, 3.6% of AF and 7.1% of CVS had non-trisomy abnormalities. There was one discrepant QF-PCR (trisomy 13)/karyotype (normal) case due to mosaicism (possibly confined to the placenta). The first year of this innovative approach to prenatal diagnosis will be discussed.

#### C09.2

#### Chromosomal abnormalities detectable by prenatal screenings cover only half of the significant fetal chromosomopathy: an evaluation based on 115'576 invasive prenatal diagnoses

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A homogeneous survey of 115'576 prenatal diagnoses have been collected from our laboratory during the last 14 years: 84'847 on amniotic fluid (AF) and 30'729 on chorionic villi (CV). This experience could be a source to draw practical and useful information since it includes a great number of women <35 years that performed invasive prenatal diagnosis without a pathologic indication (22'414 on AF and 5401 on CV). As expected, in both first and second trimester they demonstrate

an incidence of unbalanced karyotypes (+21,+18,+13, X/Y aneuploidies other than XXX and XYY, true fetal mosaicism, supernumerary markers, and *de novo* rearrangements such as deletions, duplications, reciprocal translocations, inversions) halved compared with the group with the indication "advanced maternal age" ( $\geq 35$ ). First and second trimester antenatal screenings evaluate the risk mainly for trisomy 21 and also for trisomy 13, 18, monosomy X and triploids with different detection rates (d.r.) ranging from 65% to 93%, depending on the applied test and on the chromosomal alteration. In our survey these abnormalities represent only a portion of the total fetal chromosomal pathology varying from 38.9% in women <35 in II trimester to 75.8% in those  $\geq 35$  in I trimester. Combining these data with the different reported d.r. it emerges that antenatal screenings are able to detect less than half (30.9-43.8%) of the possible fetal chromosomopathy in women <35 and, interestingly, 50.8-62.9% in those  $\geq 35$ . These findings provide the need to inform the couples about the actual limits of antenatal screenings.

#### C09.3

#### Ten years clinical application of preimplantation genetic diagnosis (PGD) for $\beta$ -haemoglobinopathies, cystic fibrosis, X-linked and rare monogenic diseases: a Greek experience

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In Greece, PGD is now an established alternative to prenatal diagnosis (PND) for couples with unsuccessful reproductive history at risk of transmitting common (CF,  $\beta$ -thalassaemia/sickle syndromes) or rare monogenic diseases. During the past 10 years we have developed several single-cell genotyping methods for detecting a wide range of genotypes associated with  $\beta$ -haemoglobinopathies, CF, Congenital Lipoid Adrenal Hyperplasia (CLAH), Glycogen Storage Disease (GSD), Leber's Congenital Amaurosis (LCA), X-linked disorders and SMA. Protocols involved denaturing gradient gel electrophoresis and more recently real-time PCR, multiplex fluorescent RLFP and/or microsatellite haplotyping. Over 10 years, requests for PGD have steadily increased, presently reaching >60 cycles annually. To date 349 PGD cycles (335 for  $\beta$ -haemoglobinopathies, 12 for CF, 1 for CLAH and 1 for GSD) have been initiated in 250 couples, with 284 cycles to oocyte-retrieval. Genotyping was performed either on blastomeres from cleavage-stage embryos (267 cycles) or trophectoderm cells from 5-day blastocysts (17 cycles). Genotypes were achieved in >80% of embryos biopsied, identifying at least one unaffected embryo for transfer in 280 cycles. One hundred and nineteen pregnancies were HCG positive, although 40 were subsequently lost spontaneously. Currently, 79 pregnancies have resulted in 102 unaffected live-births, with 10 on-going (confirmed unaffected). This cohort represents a pregnancy rate of 26.6% per oocyte retrieval or 36.25% per embryo transferred, and an embryo implantation rate of 20%. Our 10 years of experience demonstrates that PGD can be reliably incorporated within the reproductive choices for couples with unsuccessful reproductive histories at risk for transmitting serious genetic disorders.

#### C09.4

#### Evaluation and validation of Preimplantation Genetic Diagnosis (PGD) by PCR analysis: comparison of the blastomere and corresponding embryo genotype

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PGD can be an alternative for prenatal diagnosis for couples at high risk of a monogenic disorder. The aim of the present study is to validate the PGD-PCR procedure, and determine the diagnostic value. According to embryo morphology quality scores, embryos on day 4 post fertilization were divided into class 1-4, with class 4 being the lowest embryo morphology score. The genotype from the biopsied blastomere and the corresponding embryo were compared. To establish the validity of PGD-PCR procedure, sensitivity(Se), specificity(Sp), and Likelihood Ratio(LR) were calculated for the total, class 4 excluded

and class 4 embryo group. For the diagnostic value, Positive- (PPV) and Negative Predictive Value (NPV) were calculated.

In our centre 80 women underwent PGD-PCR, resulting in 793 embryo genotypes, 241 unaffected embryos were used for ET. PGD-PCR blastomere outcome, scored as affected or aberrant in 234/241 positive embryos (Se; 97.1%), and scored unaffected in 181/206 negative embryos (Sp: 87.9%). Out of the 7 false negative embryos, 6 were graded as class 4. The Se in the class 4 embryo group was 90.2% and Sp 93.2%. Exclusion of class 4 embryos resulted in Se of 99.4%, Sp of 86.4% and LR positive test of 7.3 and LR negative test of 0.006. The PPV of an abnormal PGD-PCR is 89.1%, the NPV of a normal PGD-PCR is 99.3% in this group.

PGD-PCR procedure is validated as a diagnostically reliable method for selecting unaffected embryos for ET. Accuracy of PGD-PCR analysis improves by rejecting class 4 embryos for ET.

#### C09.5

#### Multiple displacement amplification (MDA) in preimplantation genetic diagnosis (PGD)

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#### Introduction

PGD is an alternative to prenatal diagnosis, performed on single blastomeres from in vitro cultured embryos. Since many of the PGD requests for single-gene disorders involve new developments and the workup of single-cell multiplex-PCR is technically demanding and requires time and manpower, we evaluated a different approach relying on single-cell whole genome amplification (WGA) using MDA as a universal first step, followed by regular PCRs for specific loci.

#### Materials and Methods

During pre-clinical workup, MDA products from single lymphoblasts were used to assess the amplification efficiency, preferential amplification (PA), allele drop out (ADO) and contamination rates of loci (specific mutations and linked STR markers) involved in cystic fibrosis, Marfan syndrome, Huntington's disease, spinal cerebellar atrophy 7 and neurofibromatosis type 1.

Nine couples underwent 18 clinical PGD cycles, in which 100 embryos were biopsied.

#### Results

The pre-clinical results showed an amplification efficiency of 96%; no contamination was detected. This is similar to PCR-based protocols. The PA and ADO rates varied for the different loci and the average rate of 25% was five fold higher than with PCR. Still, the diagnostic efficiency in the clinical cycles was 93 %. Twenty embryos were transferred in 13 cycles, resulting in two biochemical pregnancies, one singleton baby, one twin and one pregnancy ongoing.

#### Conclusions

The relatively high ADO and PA rates associated with single-cell MDA in PGD was overcome by analysis of at least four loci. The diagnostic efficiency is similar to PCR-based protocols. These results prove the applicability of single-cell MDA in PGD.

#### C09.6

#### Fluorescence In-Situ Hybridisation using Oligonucleotide probes (Oligo-FISH): a new strategy for Preimplantation Genetic Screening (PGS).

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The principle of preimplantation genetic screening (PGS) is to biopsy 1 cell from a 6-10-cell embryo and determine chromosomal copy number within a 24 hr time frame before transferring chromosomally 'normal' embryos to the patient. Current PGS consists of sequential multicolour FISH using two or more probe sets derived from BACs. Our current clinical method consists of two rounds of FISH as follows: (1) chromosomes 13, 16, 18, 21 and 22 with a 2.5hr hybridisation time and 2) X, Y, 15 with a 16-18 hr hybridisation time allowing results to be reported in 24hr facilitating Day 4 embryo transfer. Recently FISH probes using labelled 30-mer oligonucleotides (ODNs) have been developed which

specifically hybridise to repetitive sequences on a range of different chromosomes. Based on the manufacturer's protocol, with a hybridisation step of 5 minutes, PGS for 8 chromosomes (2 x 4 chromosome probe sets) was performed in 1 hour. Four different ODNs probe sets for chromosomes (1) X, Y, 15,17 (2) X, Y, 16 (3) X, Y, 16q and (4) X, Y, 18 were validated using known normal donor lymphocytes. Validation of ODNs probe sets for chromosomes X, Y, 15,17; X, Y, 16; X, Y, 16q and X, Y, 18 scored >80% for probe efficiency using a short hybridisation time of 5 minutes. With modifications, we expect to reach > 95% efficiency for each probe set. The short length of ODN probes permits rapid hybridisation, a significant advantage for time critical procedures such as PGS.

#### C10.1

#### BRCA1 mutations and prostate cancer in Poland

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Evidence to date that BRCA1 mutation carriers are at an increased risk of prostate cancer is mixed - both positive and negative studies have been published. To establish whether inherited variation in BRCA1 influences prostate cancer risk we genotyped 1793 men with prostate cancer in Poland and 4570 controls for three founder mutations (C61G, 4153delA, 5382insC). BRCA1 mutation was present in 0.45% of cases and 0.48% of controls (OR=0.9; P=1.0). The odds ratios varied substantially by mutation. The 5382insC mutation is the most common of the three founder mutations. It was detected only in one case (0.06%), whereas it was seen in 0.37% of controls (P=0.06). In contrast, the 4153delA was more common in prostate cancer cases (0.22%) than in controls (0.04%) (OR=5.1; 95% confidence interval: 0.9-27.9; P=0.1). The C61G mutation was also found in excess in cases (0.17%) compared with controls (0.07%) (odds ratio=2.6; 95% confidence interval: 0.5-12.7; P=0.5). Eight men with prostate cancer carried a mutation. Only one of these carried the 5382insC mutation, compared with 17 of 22 individuals with mutations in the control population (P=0.003). These data suggest that 5382insC mutation is unlikely to be pathogenic for prostate cancer in Polish population. The presence of one of the other alleles was associated with an increased risk for prostate cancer (OR=3.6; 95% confidence interval: 1.1-11.3; P=0.045); in particular for familial prostate cancer (OR=12; 95% confidence interval: 2.9-51; P=0.0004). We consider that the risk of prostate cancer in BRCA1 carriers varies with the position of the mutation.

#### C10.2

#### Genomic differences between retinoma and retinoblastoma

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Genomic copy number changes are involved in the multi-step process transforming normal retina in retinoblastoma (RB) after RB1 mutational events. Previous studies on tumor samples led to a multi-step model in which after two successive RB1 mutations, the following genomic changes accompany malignancy: 1q32.1, 6p22 and 2p24.1 gains and 16q22 losses. Recent data have demonstrated that retinoma, a rare benign retinal lesion, represents an early stage in the pathway to RB. In order to catch somatic events that determine retinoma-RB transition, we investigated genomic copy number changes in DNA isolated by laser capture from retinoma and retinoblastoma tissues of two different patients. We employed both genome wide array-CGH technique and Real-Time qPCR at four genes involved in RB pathogenesis (*MDM4*,

*MYCN*, *E2F3* and *CDH11*). Our results showed that some genomic rearrangements thought to belong only to RB (Dup6p including *E2F3*, gains of *MDM4* and *MYCN*) are already present in retinoma. Tumor tissues show a higher level of genomic instability, with additional rearrangements and progressive amplification of *E2F3* and *MYCN*. Interestingly, in one of the two RB cases, we found a deletion in 16q12.1-16q12, absent in retinoma. This region includes *RBL2* (p130), an efficient inducer of cellular senescence when the major arrest pathway determined by pRb/p16INK4a is abolished. In conclusion, these data confirm the pre-malignant nature of retinoma and indicate interesting candidate genes that could have a key role in the progression to malignancy.

#### C10.3

#### High resolution analysis of chromosomal changes in colorectal tumors matched with normal tissues from the same patients using 500,000 SNPs

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Previous studies have identified many chromosomal abnormalities which occur amongst common colorectal cancers (CRCs). Until now these have been observed using fairly low resolution screening technologies so that it has been difficult to match changes to specific genes or to specific pathways. Another confounding issue has been the use of composite reference sets of "normal" DNAs to identify changes in tumor DNAs, leading to greater background noise in the estimations of copy number (CN) and loss of heterozygosity (LOH). We have used microarrays containing probes for 500,000 single nucleotide polymorphisms (SNPs) in genome wide scans to provide very high resolution estimations of CN change and LOH for each of five pairs of common colorectal tumors, pair-wise matched with normal tissues from the same patients. We show that pair-wise matching gave better definition of CN changes and regions of LOH than by comparing the tumor genome profiles against a composite genome profile derived from a reference set of 40 normal individuals. Our high resolution data allowed precise identification of many chromosomal changes in CRC tumors. We also report improved definition of some changes that have been observed previously using lower resolution methods. We show the importance of having LOH data as well as CN data to better understand the mechanisms involved in chromosomal rearrangements in CRC. These include likely instances of hemizygous deletions, gene conversions and uniparental disomy. We also present our analysis of regions gained, lost or showing LOH, that contain genes potentially involved in CRC.

#### C10.4

#### Leukemia biochip analysis of chromosomal translocations in childhood leukemia in Russia using hybridization and on-chip PCR approaches.

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Leukemia is a clinically and genetically heterogeneous disease that requires accurate molecular diagnostic approaches to generate treatment strategies and to minimize toxicity of therapies.

Here we present biochip diagnostic tool to detect 14 most significant translocations of childhood leukemia together with quantitative approach to evaluate minimal residual disease (MRD). Biochips are three-dimensional oligonucleotide microchips consisting of gel pads attached to a hydrophobic plastic surface. For diagnostics of primary leukemia, the multiplex RT-PCR was used in combination with hybridization on biochips. The quantitative method was based on real-time on-chip PCR, which allowed identification of chimeric transcript copies, as measured by PCR-synchronized fluorescent microscope. The data obtained by on-chip PCR method for t(8;21) patients was validated by conventional real-time PCR. Leukemia biochip was used to screen 753 children from newborn up to 17 years. In total of 501 primary ALL children we found translocations in 23% of cases (69 children with t(12;21) TEL/AML, 23 with t(9;22) BCR/ABL p190, 12 with t(1;19) E2A/PBX,

12 with (4;11) MLL/AF4, 4 with t(10;11) MLL/AF10, 1 with t(11;19) MLL/ELL and 1 with t(11;19) MLL/ENL); in total of 201 AML patients there were 33% with translocations (21 with t(9;11) MLL/AF9, 19 with t(8;21) AML/ETO, 16 with t(15;17) PML/RARA, 8 with inv16 and 4 with t(6;11) MLL/AF6); 24 children with CML had t(9;22) BCR/ABL p210 and of 27 with non-Hodgkin lymphomas 4 (15%) had t(2;5) NPM/ALK. In conclusion, we developed a biochip platform to diagnose primary leukemia and to monitor MRD that is fast, accurate, convenient and cost-effective.

#### C10.5

#### Disruption of Ikaros function by the CALM/AF10 fusion protein might be responsible for abortive lymphoid development in CALM/AF10 positive leukemia

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The t(10;11)(p13;q14) translocation leads to the fusion of the CALM and AF10 genes. This translocation can be found as the sole cytogenetic abnormality in acute lymphoblastic leukemia, acute myeloid leukemia and in malignant lymphomas. The expression of CALM/AF10 in primary murine bone marrow cells results in the development of an aggressive myeloid leukemia that is propagated by cells with lymphoid traits (*Deshpande et al, Cancer Cell, 2006*). Using a yeast two-hybrid screen, we identified the lymphoid regulator Ikaros as an AF10 interacting protein. Interestingly, Ikaros is required for normal development of lymphocytes, and aberrant expression of Ikaros has been found in leukemia. In a murine model, the expression of a dominant negative isoform of Ikaros causes leukemias and lymphomas. The Ikaros interaction domain of AF10 was mapped to the leucine zipper domain of AF10, which is required for malignant transformation both by the CALM/AF10 and the MLL/AF10 fusion proteins. The interaction between AF10 and Ikaros was confirmed by GST pull down and co-immunoprecipitation. Coexpression of CALM/AF10 but not of AF10 alters the subcellular localization of Ikaros in murine fibroblasts (*Greif et al, Oncogene, in Press*). The transcriptional repressor activity of Ikaros is reduced by AF10. These results suggest that CALM/AF10 might interfere with normal Ikaros function, and thereby block lymphoid differentiation in CALM/AF10 positive leukemias.

#### C10.6

#### Assessment of X Chromosome Inactivation Pattern in BRCA Mutation Carriers: Evidence for an Effect of Chemotherapy

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*BRCA1*, a major breast/ovarian cancer predisposing gene, has been suggested to play a role in the mechanisms leading to X chromosome inactivation (XCI) in female cells. In addition, a high frequency of non-random (skewed) XCI was reported in carriers of *BRCA1* mutations affected with ovarian cancer. To verify whether constitutional alterations of the gene may influence XCI status, we analyzed the occurrence of skewed XCI in blood cells from 224 female *BRCA1* mutations carriers, both with and without cancer, and 177 healthy controls. Significant reduced odds of skewed XCI respect to controls were found in younger carriers (<55 years) (OR=0.35;0.13-0.92), but not in elderly individuals (≥55 years). Using a multivariable logistic regression model, we observed that, when adjusted for age, the odds of skewed XCI in *BRCA* mutation carriers without cancer and in carriers with cancer untreated with chemotherapy, cumulatively considered, were significantly lower than in controls (OR=0.39; 0.16-1.18). Conversely, no statistically significant difference was observed in carriers with cancer who received chemotherapy (OR=1.17; 0.61-2.26). Taken together, our findings are consistent with: i) a possible selection against X skewed embryos car-

rying *BRCA1* mutation, leading to a low frequency of females with a skewed XCI respect to the general population; ii) an effect of chemotherapy on XCI status that should be taken into account when analyzing XCI in cancer patients.

### C11.1

#### Identification of causative mutations, including a ZRS sonic hedgehog regulatory variant, in an unselected cohort of 203 patients with congenital limb malformations

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Congenital limb malformations (CLMs) are common: major aetiological factors are genetic mutations and intrauterine disruptions. We have characterised the causative mutations in an unselected cohort of patients with CLMs requiring reconstructive surgery. At operation, blood was taken for DNA extraction and karyotype analysis. Candidate genes were screened for point mutations using DHPLC and sequencing, and for deletions by MLPA.

From a cohort of 203 patients, causative genetic changes were identified in 22 (11%). In addition to 4 chromosome abnormalities, these comprised mutations in *GLI3* (5), *HOXD13* (5), the ZRS of *SHH* (3), *SALL1* (2) and *SALL4*, *TBX5* and *ESCO2* (1 each). Factors that predicted the discovery of a genetic cause included bilateral malformation, positive family history, and increasing numbers of limbs affected (all p<0.01). Furthermore, 39 patients had a family history of consistent limb malformations, and 5 patients had identified syndromes, strongly suggesting a genetic basis. Therefore, at least 32% of CLMs have a genetic aetiology.

This study is the first to systematically screen for genetic mutations in an unselected cohort of patients with CLMs. Specific clinical features predict a genetic aetiology, and help to refine the selection of patients for referral to a Clinical Geneticist. Mutations of *GLI3* and *HOXD13* are particularly common causes of CLM and genetic testing is now offered by the clinical molecular diagnostic service in Oxford. In addition, we have defined a new and frequent cause of triphalangeal thumb, caused by a regulatory mutation affecting the *SHH* gene.

### C11.2

#### Genotype and phenotype of Stickler syndrome caused by mutations in the COL2A1 gene

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**Background:** Stickler syndrome is an autosomal dominant connective tissue disorder caused by mutations in different collagen genes. Characteristic clinical features include severe myopia, spontaneous retinal detachment, Pierre-Robin sequence, midface hypoplasia, sensorineural hearing loss and early-onset osteoarthritis. The aim of this study was to investigate and correlate the allelic heterogeneity and phenotypic variability in Stickler syndrome patients with a *COL2A1* mutation.

**Materials & Methods:** In 188 probands with the referring diagnosis of Stickler syndrome, the 54 exons and intronic boundaries of *COL2A1* were amplified by PCR and analysed by either a mutation scanning technique or bidirectional fluorescent DNA sequencing. The effect of splice site alterations was investigated by analysing RNA.

**Results:** We identified 100 heterozygous *COL2A1* mutations, including 1 entire gene deletion, 27 nonsense mutations, 35 frameshift mutations, 25 splice site alterations, 1 synonymous mutation altering splicing, 5 arginine-to-cysteine substitutions and 5 glycine alterations. Each of the 13 investigated splice site mutations was shown to result in a premature stop codon. A binary logistic regression analysis of the clinical features, revealed 7 major indicators for a type 2 collagenopathy in Stickler syndrome: vitreous abnormalities, retinal detachment, retinal abnormalities, low nasal bridge, cleft palate, micrognathia and positive family history.

**Conclusions:** We confirmed that Stickler syndrome type 1 is predominantly caused by loss-of-function mutations in the *COL2A1* gene and we developed a phenotypic scoring system that facilitates distinction between patients with and without a *COL2A1* mutation.

### C11.3

#### Comprehensive clinical and molecular assessment of 32 probands with congenital contractual arachnodactyly: report of 14 novel mutations and review of the literature

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**Background:** Beals-Hecht syndrome or congenital contractual arachnodactyly (CCA) is a rare, autosomal dominant connective tissue disease characterized by crumpled ears, arachnodactyly, contractures and scoliosis. Recent reports also mention aortic root dilatation, a finding previously thought to differentiate the condition from Marfan syndrome (MFS). In many cases, the disorder is caused by mutations in the fibrillin 2 gene (*FBN2*) with 26 mutations reported so far, all located in the middle region of the gene (exons 23-34).

**Methods:** We directly sequenced the entire *FBN2* gene in 32 probands clinically diagnosed with CCA. We reviewed the literature and compared the phenotypic findings of all patients harboring a *FBN2* mutation with the clinical characteristics of patients in whom no *FBN2* mutation was found.

**Results and conclusions:** In 14 probands, we found 13 new and one previously described *FBN2* mutation including a mutation in exon 17, expanding the region in which *FBN2* mutations occur in CCA.

The phenotype in *FBN2* positive patients was comparable to all previously published *FBN2* positive patients. Cardiovascular involvement included mitral valve prolapse in 2 adult patients and aortic root enlargement in 3 patients. Whereas the dilatation regressed in one proband, it remained marked in a child proband (z-score 4,09) and his father (z-score 2,94), warranting echocardiographic follow-up. We confirm paradoxical patellar laxity and report keratoconus, shoulder muscle hypoplasia and pyelo-ureteral junction stenosis as new features. In addition, we illustrate large intrafamilial variability. *FBN2* negative patients were clinically indistinguishable from patients harboring a *FBN2* mutation, suggesting locus heterogeneity.

### C11.4

#### Phenotypic Characterization of Poland Syndrome Based on a Series of 122 Patients

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Poland syndrome (PS) was first described by Alfred Poland in 1841 and classically consists of unilateral hypoplasia/ aplasia of the pectoralis muscles in isolated entity or in association with ipsilateral upper

limb or thoracic anomalies(ULA or TA).

Clinical characterization of PS has not been described in literature on a wide patient series. We have considered the following parameters:

- Describe the disease phenotype in a wide range of patients.
- Verify the current data present in literature
- Classify disease severity according to clinical features and identify risk factors according to gender, affected side and other phenotypic characteristics.
- Definition of associated malformations or syndromes.
- Obtain best methods in management of patients from diagnostic, therapeutic and prognostic points of view
- Possibility to identify new etiopathogenetic hypotheses (genetic versus environmental factors) and validate those present in literature

We have studied 122 poland patients (64 M, 48 F) in the period 2003-2007.

The management of these patients was based on multidisciplinary approach. At the first phase of the study all included patients had undergone Specialistic Counselling (Genetic, Psychologic, Surgical, and Orthopedic).

The second phase was based on medical indication and included high resolution karyotyping or array-CGH. Moreover, the standardization of pectoral muscle and tendon components by ultrasound is ongoing. Other investigations included chest X-ray, Echocardiography, Abdominal ultrasound, and thoracic CT scan.

In collaboration with AISP (Italian Association of Poland Syndrome)

A Spoken presentation on this topic was awarded young researcher prize at the Italian Society of Human Genetics Conference 2007

#### C11.5

#### **Distal limb deficiency, micrognathia syndrome (OMIM 246560) and syndromic forms of split hand foot malformation (SHFM) are caused by chromosome 10q genomic rearrangements**

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As a part of screening for genomic rearrangements in patients with unexplained syndromic limb defects, arrayCGH was performed in a cohort of patients with various syndromic limb defects. A 10q24-microduplication was detected in 6 individuals with distal limb deficiency, associated with micrognathia, hearing problems and renal hypoplasia. In addition, in a family with two affected siblings, somatic mosaicism for the 10q24-microduplication was detected in the apparently healthy mother.

This chromosomal region has previously been implicated in SHFM. SHFM3 was mapped to a large interval on chromosome 10q24. The corresponding Dactylaplasia mouse model was linked to the synteny locus on chromosome 19. When it was shown that the two existing *Dac* alleles result from *MusD*-insertions upstream of or within *Dactylyn* (*Fbxw4*), this gene seemed a plausible candidate causing SHFM3. However, all efforts to identify mutations in this gene failed. Likewise, no mutations were found in other genes within the linkage area including *FGF8*, despite the fact that the observed limb defects resemble those detected in conditional *Fgf8*-knockout mice.

However, recently, a 10q24-microduplication was detected in a total of 15 familial and 4 sporadic SHFM3 cases. In contrast to the present patients, the previously reported individuals had an isolated form of SHFM. This difference cannot be explained by a difference in size of the duplication, since a similar size was present in all individuals.

These findings extend the clinical spectrum of SHFM3. Genetic counseling should consider the observed somatic mosaicism.

#### C11.6

#### **Biallelic loss of function of the promyelocytic leukaemia zinc finger (PLZF) gene causes severe skeletal defects and genital hypoplasia**

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Deletions of 11q23 are associated with mental retardation, craniofacial dysmorphism, microcephaly and short stature. We present a patient with similar clinical findings plus absence of thumbs, hypoplasia of radii and ulnae, additional vertebrae and ribs, retarded bone age and genital hypoplasia. Using microarray based comparative genomic hybridization and microsatellite analysis, we identified an ~8 Mbp *de novo* deletion on the paternal chromosome 11, which includes the promyelocytic leukaemia zinc finger (*PLZF*) gene. In humans *PLZF* is one of five partners fused to the retinoic acid receptor alpha in acute promyelocytic leukaemia. *Plzf*-deficient mice show severe malformations of the vertebral and appendicular skeleton and male genital hypoplasia. Since patients with a deletion of 11q23 do not normally present with skeletal malformations and genital hypoplasia, we sequenced the maternal *PLZF* allele in our patient and identified a missense mutation (c.1849 A>G), which leads to the substitution of a highly conserved methionine to valine within the eighth zinc finger motive. The mutation was inherited from the mother, who does not have skeletal defects. *In vitro* reporter gene assays show that the mutation impairs the repressive function of *PLZF*. In summary, this is the first report on a germline mutation of *PLZF*. Our findings as well as observations in *Plzf*-deficient mice demonstrate that *PLZF* is a key regulator of skeletal and male germline development. Furthermore, our case highlights the importance to search for a recessive mutation on the non-deleted allele in patients with a microdeletion and atypical clinical findings.

#### C12.1

#### **Mutations in Pericentrin cause microcephalic dwarfism (Seckel syndrome) with defective ATR-dependent DNA damage signalling**

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Expansion of the brain is one of the defining characteristics of modern humans. In microcephalic dwarfism, brain and body size are markedly reduced to a similar degree to that seen in the recently discovered Indonesian hominid, *Homo Floresiensis*. Previously, only a single hypomorphic mutation in the ATR gene has been found as a cause of this genetically heterogenous group of disorders.

Here, we report that homozygous truncating mutations in pericentrin (*PCNT*) cause microcephalic dwarfism, resulting in its loss from the centrosome, where it has key functions anchoring both structural and regulatory proteins. Furthermore, we find that *PCNT*-mutated patient cells have defects in ATR-dependent checkpoint signalling, providing the first evidence linking a structural centrosomal protein with DNA damage signalling. These findings also suggest that other known microcephaly genes implicated in either DNA repair responses or centrosomal function, may act in common developmental pathways determining brain and body size, pathways potentially important in human evolution.

#### C12.2

#### **Polycomb complex shapes the higher order of D4Z4 chromatin structure during differentiation of normal and FSHD muscle stem cells**

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Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant neuromuscular disorder. FSHD involves a complex cascade of epigenetic events following contraction of a D4Z4 repeat located on chromosome 4q35.2 (FSHD locus). Previous work has indicated that

transgenic mice overexpressing FRG1, a gene proximal to the deletion, showed a phenotype resembling the FSHD disease. However, increased expression of FRG1 in FSHD patients has not been a uniform finding, and up to now several studies have failed in identifying the molecular mechanism affecting the FSHD locus functionality. We took advantage of ChIP/MeDIP and 3D immuno-FISH assays as complementary approaches to depict the higher order of chromatin organization of 4q35.2 region during myogenic differentiation of healthy and FSHD myoblast and mesoangioblast stem cells. We found that FRG1 undergoes to muscle specific regulation through a two-step activation mechanism, whereby removal of H3-K27 methylation and Polycomb complex components precedes MyoD recruitment on the FRG1 promoter; intriguingly, the same chromatin structure and Pcg recruitment were contemporaneously found on D4Z4 array, rendering the Polycomb complex the first molecular player that links FSHD locus to myogenic differentiation. Moreover, D4Z4 H3-mK27 signals were strongly reduced in FSHD myoblasts in respect to controls, suggesting the severe impairment of the Pcg complex recruitment. Nevertheless, molecular alterations of the D4Z4 array do not have in FSHD myoblasts an effect in cis on FRG1 gene expression. These observations evidence a role of 4q35 D4Z4 in muscle differentiation, probably through inter-chromosomal interactions.

### C12.3

#### Active transport of the ubiquitin ligase MID1 along the microtubules is regulated by protein phosphatase 2A

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Mutations in the MID1 protein have been found in patients with Opitz BBB/G syndrome (OS), which is characterised by multiple malformations of the ventral midline. MID1 is a microtubule-associated protein that stabilizes microtubules and, in association with the regulatory subunit of protein phosphatase 2A (PP2A),  $\alpha 4$ , provides ubiquitin ligase activity for the ubiquitin-specific modification of PP2A. Using Fluorescence Recovery After Photobleaching (FRAP) technology, we show here that MID1 is actively and bi-directionally transported along the microtubules, and that this movement is directly linked to its MAP kinase and PP2A-mediated phosphorylation status. Intact transport depends on both kinesins and dyneins and is inhibited upon taxol and colcemide treatments. MID1 proteins carrying missense mutations in the  $\alpha 4$  binding domain still bind the microtubules but can not be actively transported. Likewise, knock-down of the  $\alpha 4$  protein, inhibition of PP2A activity by okadaic acid and fostriecin or the simulation of permanent phosphorylation at Ser96 in MID1 stop the migration of MID1-GFP, while preserving its microtubule-association. In summary, our data uncover an unexpected and novel function for PP2A, its regulatory subunit  $\alpha 4$  and PP2A /  $\alpha 4$  / mTOR signalling in the active transport of the MID1 ubiquitin ligase complex along the cytoskeleton. Furthermore, a failure in the microtubule directed transport of this protein complex would be an attractive mechanism underlying the pathogenesis of OS in patients with B-box1 mutations.

### C12.4

#### A centrosomal protein molecularly links Usher syndrome to Leber congenital amaurosis and Bardet-Biedl syndrome in the retina

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Usher syndrome (USH) is the most common cause of hereditary deaf-blindness in man. Ten loci are known for Usher syndrome, and we and others provided evidence for the existence of a protein network of the USH proteins at different subcellular sites in the retina and inner ear.

Disruption of one of the members of the USH network can lead to malfunction and degeneration of both photoreceptor cells and hair cells.

To elucidate the pathogenic mechanisms of Usher syndrome, we searched for novel interacting partners for the intracellular region of USH2A isoform B. This revealed the interaction with a centrosomal protein. Interestingly, simultaneous screens for interactors of the recently identified lebercillin (LCA5, and associated with Leber congenital amaurosis, LCA), identified the same centrosomal protein. In order to clarify the role of this protein *in vivo*, knockdown studies in zebrafish were performed. This gave rise to a classical planar cell polarity phenotype, similar to the defects observed after knockdown of the genes involved in Bardet-Biedl syndrome (BBS). Yeast two-hybrid interaction analysis subsequently revealed a specific physical interaction between the centrosomal protein and BBS6.

Our data indicate that the same centrosomal protein interacts physically with USH2A, lebercillin and BBS6, thereby linking the retinal ciliopathies Usher syndrome, Leber congenital amaurosis and Bardet-Biedl syndrome at the molecular level. The physical and genetic interactions between proteins/genes involved in Usher syndrome and Bardet-Biedl syndrome suggest a putative role for the Usher interactome in the establishment of planar cell polarity, with a central role of the cilia.

### C12.5

#### Study of the role of the Ofd1 transcript in limb patterning and endochondral bone development

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Oral-facial-digital type I (OFDI) syndrome is an X-linked dominant male lethal developmental disorder characterized by oral, facial and digital abnormalities. Recent data ascribed OFDI to the growing number of diseases due to dysfunction of primary cilia. Ofd1 null mutants recapitulate the phenotype observed in OFDI patients and displayed skeletal defects. To overcome the embryonic male and perinatal female lethality observed in Ofd1 null mutants we have generated a conditional model with Ofd1 limb mesenchyme specific inactivation. These mice displayed a severe polydactyly with loss of antero-posterior digit patterning, aberrant cilia formation, and shortened long bones. Defective digit patterning was found to be associated to progressive loss of Shh signaling and impairment of Gli3 processing. Shortening of long bones was found to be associated to misregulation of Ihh expression and activity during endochondral bone formation as revealed by RNA *in situ* studies. Immunoistochemical analyses to assess the proliferative state of chondrocytes revealed an increase in the number of proliferating pre- and hypertrophic chondrocytes in male mutants. This data suggest that the shortening of long bones observed in Ofd1fl/flPrx1Cre mice is likely due to an increase in the number of proliferating chondrocytes associated to a delay in terminal chondrocytes differentiation. Finally Von kossa staining and RNA *in situ* studies demonstrated defective bone mineralization accompanied by loss/reduction of bone collar development suggesting a defect in osteoblast differentiation.

Altogether our data demonstrate that Ofd1 is a patterning factor that plays multiple roles in limb and endochondral bone development.

### C12.6

#### Integration into molecular diagnostic procedures of systematic screening for sequence variants of unknown significance using a splicing reporter minigene

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Unclassified variants (UVs) found in genes involved in genetic diseases may have an effect on pre-mRNA splicing. In clinical practice, the interpretation of UVs is limited because patient blood samples are often not suitable for RNA analysis. We have recently developed a screening strategy based on genomic DNA from patients, using a splicing reporter minigene. We have now applied this screening protocol to more than 150 UVs from many genes including *MSH2*, *MLH1*,

*APC*, *BRCA1* and *BRCA2*. The protocol steps have been streamlined for routine application as follows: 1) PCR amplification of the relevant exon, including flanking intronic sequences, 2) cloning into a two-exon splicing reporter minigene, 3) selection of wild-type- and variant-carrying plasmids, 4) transfection into HeLa cells, 5) RNA extraction and RT-PCR using primers targeting minigene sequences, and 6) sequence analysis of all RT-PCR products. Variants associated with total absence of correct exon inclusion in this monoallelic assay are considered as most likely pathogenic but UVs inducing partial alterations of normal mRNA sequences are also considered as biologically significant. In spite of our initial concern that exons are tested in a heterologous context, extensive comparisons with *in vivo* RNA data have shown that this minigene-based approach is sensitive and specific. Comparisons with results from *in silico* analysis indicate that current bioinformatic predictions are sensitive and rather specific concerning changes in splice site strength, activation of cryptic sites or generation of new splice sites, but are still inadequate for predicting the presence of relevant exonic splicing regulatory elements.

### C13.1

#### A genome-wide scan of adult human stature and skeletal size

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Human adult stature is a classical quantitative trait and a paradigm for genetic association studies of quantitative trait variation. We have carried out a meta-analysis of four genome-wide association scans of stature produced using the Illumina HumanHap300 SNP panel in 10,050 adults from four population-based cohorts (TwinsUK, EPIC Norfolk and 1958 Birth Cohort from the UK and the Rotterdam Study from the Netherlands). We have identified eighteen loci showing association with height with P-values of less than  $10^{-5}$ , which we have brought forward for replication in an independent sample of 9,000 individuals.

The signals identified provide strong evidence for replication in genomic regions previously implicated in height, including HMGA2 (rs8756, P-value =  $5 \times 10^{-13}$ ) and GDF5-UQCC (rs4911494, P-value =  $1.5 \times 10^{-10}$ ). In addition, we have identified novel candidate genetic loci for human height, some of which are in or near genes implicated in cellular growth and development (HHIP, ADAMTSL3 and DLEU7). In an attempt to dissect the mechanisms underlying human growth, we have tested the association of these novel candidate height loci with different measurements of skeletal growth. Our results provide both novel and confirmatory evidence for the implication of genes and pathways in human growth, thus contributing to the understanding of the biological processes underlying many common and severe human diseases.

### C13.2

#### Super-hotspots for Meiotic Recombination in the Human Genome

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Homologous recombination is a vital process for ensuring proper chromosome segregation during meiosis, as well as increasing diversity by reshuffling haplotypes between generations. In this study, we analysed Phase II HapMap data to identify autosomal regions showing extreme breakdown of linkage disequilibrium (LD). Sixteen of these regions were selected for crossover analysis directly in sperm. All contained active sperm hotspots, with similar characteristics as at previously studied hotspots, i.e. normally-distributed crossover breakpoints within regions 1-2 kb wide. These new hotspots were on average 10 fold more active than previously characterised autosomal hotspots and include the most active crossover hotspots yet discovered in the human genome. Their activity is however poorly predicted from LD data. Most crossovers in these hotspots were simple, exchanging haplotypes within a single interval between markers. However, 0.3% of exchanges were more

complex, switching haplotypes at several intervals during an exchange event. Most of these occurred within the boundaries of the hotspot while 22% occurred beyond the hotspot, implying a broader region involved during intermediate stages of recombination. Several hotspots showed crossover frequency variation between men, including two cases of complete presence/absence polymorphism. Instances of extreme or subtle biased gene conversion accompanying crossover were observed within some hotspots, in some cases correlating with crossover frequency variation between men. Curiously none of the most active hotspots showed polymorphism or strongly biased conversion, in contrast to the prediction that these hotspots should be the most vulnerable to attenuation/extinction by meiotic drive in favour of recombination suppressors.

### C13.3

#### A full survey of common copy number variation in the human genome

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Copy number variation (CNV) in the genome is extensive and yet is grossly under-ascertained. As smaller CNVs are expected to be far more numerous than larger CNVs, improved CNV detection resolution will dramatically increase the numbers of known CNVs. The Genome Structural Variation Consortium has performed comparative genome hybridisation on a genome-wide set of tiling oligonucleotide arrays to discover the majority of common copy number variants >500bp in size in two populations with African and European ancestry. This set covers the assayable portion of the human genome with 42,000,000 probes with a median spacing of ~50bp. In addition we have generated data on a single chimpanzee to provide information on the ancestral state of observed variants. The results reveal, as expected, that previous surveys captured only 5-10% of the CNVs within a single genome. Because the boundaries of thousands of CNVs are defined precisely by this probe set, we can identify accurately functional sequences included in copy number variable regions. This provides new insights into the mechanisms generating chromosomal rearrangements and the biological functions of common CNVs.

### C13.4

#### Gene expression variation from peripheral blood in the general population - the KORA study

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Interrogation of gene expression variation in humans will provide better understanding of functional genetic variation and help identify disease variants. At present, the nature and extent of variation in transcript levels across the entire genome is largely unknown.

We assessed normal variation in gene expression from 350 KORA individuals, using the Illumina Human Ref-6 v2 whole genome microarray. Special features of inter individual variation in peripheral blood expression could be traced to gender and age differences. Several significant age-related genes and distinct gender-specific gene signatures were identified. Using the PAM algorithm, it was possible to predict the gender with an accuracy of 98%.

Using available KORA Affymetrix 500k genotypes, we performed a genome-wide association study to compare peripheral blood eQTLs (Expression Quantitative trait loci) to published lymphocyte cell culture eQTLs.

Expression data can be used to prioritize candidate genes with expression levels significantly correlated to the trait. In this context, a recent genome wide association study using the KORA population found the most significant SNPs associated with urate levels mapped within an uncharacterized carrier gene SLC2A9. Our analysis revealed a significant association between SLC2A9 expression and urate levels (Doering et al, Nature Genetics in print).

Analysis of further candidate genes where genome-wide association signals have been obtained is currently underway.

**C13.5****The genetic control of microRNA expression variation in Humans**

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

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate the expression of protein-coding. Each miRNA is thought to have multiple target genes that are regulated at the post-transcriptional level. Inter-individual variation of miRNA gene expression is likely to influence the levels of target genes, and may therefore contribute to some phenotypic differences, including susceptibility to common disorders. The aim of this study was to characterize the natural variation in miRNA expression levels present in different individuals, and to identify loci that control this variation.

We established primary fibroblasts from 200 unrelated umbilical cord samples of Caucasian origin (GenCord collection). All of these samples have been genotyped using the Illumina Hap550 SNP array. After multiple filtering steps 433'000 were retained for statistical analyses. Taqman real-time PCR in all cell lines was used to measure the expression of 365 known mature microRNAs in each sample. This revealed substantial differences in miRNA expression levels between individuals, which were up to 50-fold in some cases. Normalized miRNA levels for each individual were used to perform quantitative whole genome association studies using the Plink software. We will present the detailed genome-wide association analysis of SNPs that control in cis- or trans- 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.

**C13.6****Comparison of different methods to estimate genetic ancestry and control for stratification in genome-wide association studies**

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In case-control association studies, population subdivision or admixture can lead to spurious associations between a phenotype and unlinked candidate loci. Population stratification can occur in case-control association studies when allele frequencies differ between cases and controls because of ancestry.

We evaluated five methods (Fst, Genomic Control, STRUCTURE, PLINK and EIGENSTRAT) using 317K SNPs (Illumina HumanHap300) in a case-control sample of 200 American subjects with different races (Caucasian, African and Asian) in order to identify and to correct for stratification. Fst, Structure and Genomic Control are based on the usage of few genetic markers while PLINK and EIGENSTRAT are computationally tractable on a genome-wide scale. Fst, STRUCTURE and Genomic Control did not detect a significant stratification in our sample, as well as EIGENSTRAT and PLINK. However, these last two methods, using a much larger information from the whole set of SNPs, graphically suggested the presence of a partial stratification, due to African and Asian individuals while the estimated inflation factor of 1 didn't statistically confirm stratification. This brought to the decision to further enlarge the sample with hundreds of controls coming from Caucasian populations. When we enlarged the sample to 650 individuals we found a high value of inflation factor as statistical confirmation of the population stratification. The substructure still depends only on African and Asian subjects that are separated from the Caucasian homogeneous sample. Therefore the sample size is crucial to get enough power to detect a possible stratification.

**C14.1****The ethics of undertaking research in other countries**

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There are many reasons for scientists to undertake research with colleagues in other countries: to share knowledge and experience with

colleagues; to obtain funding directed to transnational projects; to gain access to facilities and research participants; to acquire kudos, academic advancement, or commercial benefits; or to undertake activities that would not be permitted in their own country, due to legal or ethical constraints.

This paper considers the last reason and when it is ethical for scientists to do research abroad that is banned at home. The author argues that such research may sometimes be ethical, provided it is scientifically rigorous and accords with international ethical principles and ethical oversight requirements. But it is not ethical when the proposed research is widely regarded as unethical in the home country. The author uses her own image of Skene's *Ethico-Legal Barometer* to gauge the activities that attract this degree of sensitivity and would be unethical wherever they are done. This research is in the red zone of the barometer and the closer an activity falls to the red zone, the greater the need for ethical review and oversight before being ethically acceptable in the home country.

The author illustrates these arguments with examples like human embryonic stem cell research, somatic cell nuclear transfer and research involving human subjects.

**C14.2****Direct-to-consumer services. A review of the debate**

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Background. The rapid expansion of the internet has meant that there is now a global marketplace for a multitude of health care products and services that are available directly to the consumer. Related to this, in the field of genetics, the rapid expansion of knowledge about the relationship between genetics and human disease has set the groundwork for commercial enterprises that use direct-to-consumer (DTC) advertising for genetic tests and, in some cases, bypass the supervision of a health professional to offer DTC purchase of the tests. Various companies (e.g. Sciona (UK), DNA direct (US), Genelink (US), Test Kimball Genetics (US), Geneticom (The Netherlands)...) have been identified where direct-to-consumer tests are offered (e.g. for paternity testing, ancestry testing, susceptibility tests for cardiovascular diseases, hereditary hemocromatosis, osteoporosis, Factor V Leiden, type 2 diabetes...).

Objective. This paper wants to investigate the existing ethical and legal framework of direct-to-consumer services.

Method. It will offer a review of the debate through a systematic analysis of the position papers, reports, guidelines or statements emanating from international and national organisations, bioethics committees, and professional associations, together with the academic literature identified after an extensive literature search.

Results. This paper offers an overview of the debate. It identified weaknesses in the existing regulatory framework and suggested further pathways for research.

**C14.3****Preventive genetic screening in the isolated community: lessons learned**

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In the countries with high rates of consanguinity, many inherited diseases are present with a high frequency only in a limited geographical region. We present a comprehensive strategy for genetic diseases prevention program in the isolated community in Israel and examine the impact of the screening on the population.

During years 2003-2007, we carried out carrier screening among the residents of an isolated village of 10,500 inhabitants with a high frequency of non-syndromic mental retardation (MRT3), spinal muscular atrophy, spinal muscular atrophy with respiratory distress and cystic fibrosis. The subjects were pregnant women visiting the women's health station for routine monitoring. The medical geneticist or genetic counselor provided counseling. A three-generation pedigree was constructed by interviewing the women and the nurses. The screening was provided free of charge, financed by the Israeli Ministry of Health. We

identified 215 carriers for one of the diseases and 14 carrier couples. Carrier frequency for MRT3, SMA, SMARD and cystic fibrosis was 1:11, 1:13 and 1:10 and 1:21, respectively. Among carrier couples, 15 pregnancies were recorded and 2 pregnancies of affected fetuses terminated. More than 50% of carrier couples accepted prenatal testing. While the women in the village were willing to collaborate, an increase in the willingness to collaborate among men was observed gradually. The availability of genetic counseling locally and education of the population are essential for the success of the prevention program. Ethical aspects of preventative programs based on genetic screening of premarital, pre-conceptual couples or couples during pregnancy will be discussed.

#### C14.4

##### Differences and similarities in breast cancer risk assessment models in clinical practice: which model to choose?

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**Background:** Models regarding breast cancer risk assessment focus on family history and some use personal risk factors additionally. Aim of this study is to show differences and similarities between the different models in risk estimates for breast cancer in healthy women from BRCA1/2-negative or untested families.

**Methods:** After a systematic literature search seven models were selected: Gail-2, Claus Model, Claus Tables, BOADICEA, Jonker Model, Claus-Extended Formula, and Tyrer-Cuzick. Life-time risks (LTRs) for developing breast cancer were estimated for two healthy counselees with variety in family histories and personal risk factors. The estimated LTRs and the threshold for individual mammographic screening based on guidelines were compared.

**Results:** Without a clinically significant family history LTRs varied from 6.7% (Gail-2 model) to 12.8% (Tyrer-Cuzick Model). For counselees with low and moderate risk, the models mostly agreed. Difficulties in screening decisions were encountered in some moderate and high risk individuals, and when including personal risk factors into the estimations.

**Conclusion:** Older models (i.e. Gail-2 and Claus) are likely to underestimate the LTR for developing breast cancer as their baseline risk for women without a significant history of breast cancer is too low. Current guidelines have been formulated on breast cancer risks based on family history alone. When models include personal risk factors, surveillance thresholds have to be reformulated as other factors are applied. For current clinical practice, the Tyrer-Cuzick Model and the BOADICEA Model seem good choices.

#### C14.5

##### Treatable and untreatable diseases in the neonatal-screening programme: the opinion of future parents in The Netherlands

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In the Netherlands, in 2007, the neonatal screening program was expanded from 3 to 17 disorders for which screening met the Wilson and Jungner criteria, especially regarding treatability. The decision whether or not to add diseases to the newborn screening programme has often been made by expert groups, whereas the opinion of those whom it concerns: the parents-to-be remains unknown. In our research we investigated the opinion of future parents concerning screening newborns also for incurable, but to some extent treatable or even untreatable disorders.

A structured questionnaire consisting of 3 parts in which similar questions were posed about treatable, incurable, but treatable, and untreatable childhood onset disorder was posted on the website of a national pregnancy fair. 1631 prospective parents filled out the questionnaire. 259 were excluded because they did not meet our inclusion criteria. In contrast to current policy, overall they showed a positive attitude towards inclusion of incurable, but treatable [88%] or non-treatable disorders [73%] within the national newborns screening programme. Respondents who already had children at the time of filling out the questionnaire were even more in favour of uptake of childhood dis-

orders, especially untreatable diseases. The most important reason mentioned was: to prevent a long diagnostic quest. Obtaining information to enable reproductive choices in future pregnancies was hardly mentioned.

Since a relevant part of the Dutch population seems interested in considering screening newborns for untreatable disorders we argue that further debate is needed between policy, public and health care professionals to discuss pros and cons.

#### C14.6

##### Promoting clinically relevant genetics education for medical trainees: the importance of educational outcomes and resources for each stage of medical training

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It is widely acknowledged that the genetics education of healthcare staff across the world has not kept pace with advances in genetics. The NHS National Genetics Education and Development Centre is working to address this situation in the UK by promoting clinically relevant genetics education for healthcare professionals across all stages of education and training. To achieve this, it is important to develop clinically relevant educational outcomes and to identify and develop resources to support these across all stages of training. This will be illustrated by results from the UK.

The first step in promoting clinically relevant genetics education was to develop educational outcomes linked to clinical practice at each stage of training. Outcomes have been developed for medical students and general practice and specialty trainees by working with health professionals to define genetics relevant to practice for these stages of training. To promote incorporation of these outcomes into curricula, the Centre worked with education bodies to raise awareness of the importance of clinically relevant genetics education.

Now the outcomes have been incorporated into medical training curricula, the Centre is focusing on supporting teachers and learners through the development and evaluation of learning and teaching resources and professional development for educators. Resources covering core genetics concepts for medical students and resources to support general practice training are currently being developed.

The educational outcomes and many of the resources will be made freely available online at [www.geneticseducation.nhs.uk](http://www.geneticseducation.nhs.uk) and may be of use to those involved in genetics education across Europe.

#### C15.1

##### Systemic antisense-mediated exon skipping studies in mouse models for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe, progressive neuromuscular disorder leading to loss of muscle function and generally premature death before the age of 30. The disease is caused by mutations in the *DMD* gene that disrupt the open reading frame and lead to complete abolishment of functional dystrophin. Our group has pioneered antisense-based exon skipping to restore the reading frame. Here, antisense oligoribonucleotides (AONs) induce specific exon skipping during pre-mRNA splicing. They have been successful in repairing the disrupted open reading frame and the generation of internally deleted, partially functional Becker-like dystrophins. Proof of concept has been achieved in cultured muscle cells from patients and the *mdx* mouse model. Recently, exon 51 skipping and dystrophin restoration was confirmed after a single intramuscular dose of AON in a local-administration clinical trial in 4 patients. Our current research focuses on optimizing systemic delivery of 2'-O-methyl phosphorothioate AONs and comparison of different routes of administration and dosing regimes. We show that after systemic injection, AONs are preferentially taken up by dystrophic muscle compared to healthy fibers. Furthermore, we were able to induce exon skipping and dystrophin restoration in all muscles, including the heart after short term treatment with high AON

doses and long term treatment with lower doses. This was accompanied by functional improvement and improved muscle integrity, without any apparent toxicity. These findings are encouraging for future clinical trials and eventual systemic application of this approach.

#### C15.2

#### Restoration of aberrant splicing and neurofibromin function in three NF1 deep intronic mutations by antisense morpholino oligonucleotides (AMOs)

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder caused by mutations in the *NF1* gene. Approximately 2% of the germline mutations identified in our population consists in deep intronic mutations. Such nucleotide changes create new splice sites that produce the insertion of a cryptic exon in the mature mRNA. We used antisense morpholino oligonucleotides (AMOs) to restore normal splicing in three *NF1* deep intronic mutations (c.288+2025T>G, c.5749+332A>G and c.7908-321C>G). All of them generate a cryptic 5' splice donor site and result in the inclusion of a cryptic exon in the mature RNA by the use of an existent 3' cryptic splice site. AMOs were designed to target the newly created 5' splice sites in order to avoid the incorporation of cryptic exons and promote the use of wild-type splice sites, by the splicing machinery. Our results demonstrate that AMOs treatment effectively restore normal *NF1* splicing at the mRNA level in primary fibroblast and lymphocyte cell lines derived from different patients carrying the three deep intronic mutations. In addition, we observed a decrease in the amount of Ras-GTP (equivalent to wild type fibroblast levels) in primary fibroblasts from patients after AMOs treatment, consistent with the restoration of neurofibromin function. To our knowledge this is the first time that an antisense technique is used successfully to restore *NF1* mutations, opening the possibility of a therapeutic strategy for this type of *NF1* mutations.

#### C15.3

#### Antisense therapeutics for a new deep intronic variation identified in two Methylmalonic Acidemia patients

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Isolated methylmalonic acidemia (MMA) is a life threatening organic acidemia caused by defects in the methylmalonylCoA mutase (MCM) or in enzymes involved in the synthesis of the active cofactor adenosylcobalamin. In this work we describe a new point change identified in two MMA affected patients located deep in intron 11 of the MUT gene (IVS11-898A>G). This change increases the splicing score of a 5'cryptic splice site and provokes the intronic inclusion of 76 bp (r.1957ins76) between exons 11 and 12. Using a splicing assay we have demonstrated that the change caused exonization of this intronic sequence and by morpholino antisense oligonucleotide transfection we have demonstrated that the insertion is a disease-causing mutation in these two patients. The antisense oligonucleotide was targeted to the 5' cryptic splice sites to block access of the splicing machinery to the pseudoexonic region in the pre-mRNA. After transfection of the patient's fibroblasts we have performed RT-PCR analysis and enzymatic assay to determine MCM activity. Using this antisense therapeutics we have obtained correctly spliced mRNA that was effectively translated and methylmalonyl CoA mutase activity was rescued in patient's fibroblasts close to 100% of control activity. The effect of AMO is sequence and dose dependent and was not effective in patients where the insertion was produced by splicing background noise. These findings add to previous results providing a new therapeutic strategy in this genetic disorder and potentially applicable to large numbers of cases with deep intronic changes that, at the moment, remain undetected by standard mutation-detection techniques

#### C15.4

#### Rescue of a Lethal Murine Model of Methylmalonic Acidemia using AAV 8 Mediated Gene Therapy

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Methylmalonic acidemia (MMA), a severe organic acidemia, is caused by deficient activity of the ubiquitous mitochondrial enzyme methylmalonyl-CoA mutase (MUT). MMA patients exhibit increased methylmalonic acid levels in the plasma, urine and CSF and display a clinical phenotype of lethal metabolic decompensation, growth retardation, renal failure and metabolic strokes. To assess the potential of genetic therapy for MUT MMA, we employed a mouse model of MMA that produces no detectable Mut transcript or protein. AAV 8 CBA-Mut was injected directly into the liver of newborn Mut-/- pups. Currently, 28 out of the 29 Mut-/- mice injected with 1 or 2x1011GC of AAV 8 CBA-Mut are alive beyond DOL 90 with some treated Mut-/- mice older than 200 days. All the untreated mutants (n=21) perished before DOL 72. The treated Mut-/- mice are thriving and indistinguishable from their wild-type (WT) littermates. AAV 8 CBA-Mut treated Mut-/- mice achieved body weights comparable to controls while untreated mutants experienced post-natal growth retardation and reached only 40% of the weight of the WT. Plasma methylmalonic acid levels in the treated mutant mice on an unrestricted diet were significantly reduced compared to uncorrected animals, indicating that substantial Mut enzymatic activity was restored after AAV therapy. At DOL 90 the liver from a treated Mut-/- mouse had WT levels of Mut protein by Western blot analysis. These experiments provide the first evidence that gene therapy has clinical utility in treatment of MMA and support the development of gene therapy for other organic acidemias

#### C15.5

#### Evaluating suppression of nonsense mutations by aminoglycoside antibiotics as an intervention for vision loss in type I Usher syndrome

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Type 1 Usher syndrome (USH1) is a recessively-inherited condition, characterized by profound prelingual deafness, vestibular areflexia, and prepupal onset of retinitis pigmentosa (RP), which to date has no effective treatment. USH1 can be caused by mutations in each of at least six genes. While truncating mutations of these genes cause USH1, missense mutations of some of the same genes cause nonsyndromic deafness, suggesting that partial or low level activity of the encoded proteins may be sufficient for normal retinal function, although not for normal hearing. Interventions to enable at least some translation of full-length protein, may delay the onset and/or progression of RP in individuals with USH1 due to nonsense mutations. One such possible therapeutic approach is suppression of nonsense mutations by aminoglycosides. We demonstrated up to 91% suppression of *PCDH15* nonsense mutations by commercial aminoglycosides *in vitro*. We also demonstrated *ex vivo* suppression, by the same aminoglycosides, of the R245X mutation. We are now testing suppression of several *CDH23* nonsense mutations. In parallel, we are developing a series of new aminoglycoside-derived compounds, which includes two new promising derivatives, NB30 and NB54. Based on cell toxicity assays and on acute toxicity measurements in mice, the toxicity of both compounds is significantly reduced, in comparison to commercially available aminoglycosides. Based on *in vitro* and *ex vivo* experiments, their suppressive activity is maintained. The research described here will have important implications for development of targeted interventions that are effective for patients with USH1 and nonsyndromic RP caused by various nonsense mutations.

## ESHG POSTERS

## P01. Clinical genetics

## P01.001

## Most Encountered Genetic Disorders in Egypt: Classification &amp; Registry

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Diseases with genetic bases have been a major health problem to every society. Heavy economic, social and health burdens are imposed on the afflicted family as well as the society. In general genetic diseases are relatively prevalent among the Arab population. Incidence of congenital malformations among Egyptians ranges from 1,16 to 3,17 %. This is probably due to the high consanguinity rate (20 - 40 %). Early diagnosis of various genetic disorders with proper intervention will reduce the burdens of genetic disorders at the all levels.

A comprehensive classification system is necessary for genetic diseases in order to provide a framework in which to study the etiology, pathogenesis and treatment of diseases in an orderly fashion. Such system gives clinical geneticists a way to organize the health care needs of their patients. We revised different classifications to determine which classification to follow. However these classifications were based on the etiological diagnosis, pathological diagnosis, phenotypic diagnosis and / or mode of inheritance. Therefore, we established our own classification, as a modification of the previously mentioned. The main purpose of our classification is to include four major descriptive categories (axes), that geneticists consider to identify the genetic disorders. The Final Report of the study (1/7/2004 - 30/6/ 2007) included 3417 cases. We established an integrated classification for the genetic disorders referred to Genetic Clinic. This classification considers the etiological, phenotypic, differential diagnosis and referral axes& is entitled "Genetic/Diagnostic/Referral Classification

## P01.002

## Genetics Epidemiology Study of Bashkortostan Republic

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The results of genetic epidemiological study of five Districts (Burzyansky, Baimaksky, Abzelilovsky, Salavatsky and Archangelsky) of Bashkortostan Republic are reviewed. The total size of the investigated population was 168050 persons, including 135748 southern- east and northern-east ethnographic group the Bashkir. Medical genetic research included all population of five districts, indigenously of a nationality and was carried out under the standard report developed in laboratory genetic epidemiology Research Centre for Medical Genetics. Segregation analysis demonstrated good agreement between the observed and expected segregation frequencies for both AR and AD diseases. The prevalence rates of hereditary disorders (autosomal dominant, autosomal recessive and X-linked) for urban and rural, Bashkirs and other ethnic groups were calculated. Significant differences in the prevalence rates were revealed between the prevalence rates AD and AR disorders in rural and urban populations. The prevalence rare for AD and AR disorders was twice lower in the urban populations than those in the rural ones . The prevalence rate of all Mendelian disorders varied in the investigated populations from 1.54 in Baymak city from 6.12 per 1000 persons in Burzyansky District. Spectrum of AD diseases consisted of 83, spectrum of AR diseases - 48 nosological forms, and X-linked - 13 forms.

## P01.003

## The National Register of Congenital Malformations in Moldova: Comparative Analysis for Years 2005-2007

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Objectives. Since 1989 conform to Order of Ministry of Health N-129 from 27.04.89 was introduced a national system of monitoring of con-

genital malformations (CM). Assistance in creation of modern National register of Congenital malformations and hereditary disorders corresponding to European standards.

Methods. Were obtained 747, 598 and 453 questionnaires for the period of 2005 - 2007, respectively. Were used standard methods of statistical analysis.

Results. The prevalence of CM per 10000 births decreased from 2005 to 2007 and was 199.29 in 2005, 160.00 in 2006 ( $X^2=15.795$ , OR=1.246,  $p<0.001$ ) and 118.96 in 2007 ( $X^2=22.196$ , OR=1.345,  $p<0.001$ ). We are investigated changes in prevalence of sentinel CM. In 2006 decreased frequency of anal atresia (4.80 v. 1.07,  $X^2=7.642$ , OR=4.487,  $p=0.006$ ), esophageal atresia (6.40 v. 1.07,  $X^2=12.833$ , OR=5.983,  $p=0.003$ ), limbs reduction (7.20 v. 0.54,  $X^2=19.785$ , OR=13.461,  $p<0.001$ ), polydactilia (14.41 v. 2.67,  $X^2=28.741$ , OR=5.384,  $p<0.001$ ), omphalocele (14.41 v. 3.21,  $X^2=28.741$ , OR=5.384,  $p<0.001$ ), Down syndrome (25.61 v. 6.42,  $X^2=41.735$ , OR=3.989,  $p<0.001$ ) and multiple CM (22.41 v. 10.70,  $X^2=14.762$ , OR=2.094,  $p<0.001$ ). In 2007 decreased frequency of omphalocele (3.20 v. 0.54,  $X^2=4.688$ , OR=4.545,  $p=0.030$ ), dysplasia of hip joints (36.55 v. 12.31,  $X^2=20.146$ , OR=2.256,  $p<0.001$ ), and increased frequency of polydactilia (2.67 v. 5.08,  $X^2=4.888$ , OR=0.399,  $p=0.030$ ).

Conclusion. The data from Moldavian Registers are valid statistical tool to make surveillance of CM in the region. Cooperation with EUROCAT register can improve planning of medical service in Moldova and will enlarge European database of CM cases.

## P01.004

Sensitivity and Specificity of MCV Test for Screening of  $\alpha$  and  $\beta$  Thalassemia traits

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Aim: To evaluate the sensitivity and specificity of mean corpuscular volume (MCV) for screening of both Alpha and Beta thalassemia trait

Method: Diagnostic test was conducted on 1500 patients; MCV was measured in all samples using an automated hematology Analyzer [sysmexkx21]. HemoglobinA2 measured by column chromatography. Cut off value for HbA2 was 3.5%. PCR tests were done in all cases for detecting about 4 different deletional mutations in  $\alpha$ - globin genes such as-  $\alpha^{3.7}$ , -  $\alpha^{4.2}$ ,

-  $\alpha^{20.5}$ , and --MED for other non detectable mutation we used sequencing and analysis about 25 different mutation. MCV $\leq$ 80 fl showed a sensitivity of 93.5% and specificity 91.5%.

Conclusion: MCV evaluation is a useful tool for screening of alpha thalassemia and  $\beta$  thalassemia traits because of its simplicity, low cost and high sensitive.

## P01.005

Molecular Characterization of Two Families With  $\delta\beta$  Thalassemia

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$\beta$ - thalassemia is the most common genetic disorder in Iran, caused by reduced or absence of globin gene synthesis in  $\beta$ -globin chain. More than 180 different mutations in the  $\beta$ -globin gene have been reported. Most of these mutations are point mutations, and large deletions are not common.  $\delta\beta$ thalassemia normally results from deletions involving either the  $\delta$ , $\beta$  globin genes or the Ay,  $\delta$  and  $\beta$  genes. It is useful to broadly divide  $\delta\beta$ -thalassemia in to the  $(\delta\beta)^+$ and $(\delta\beta)^0$  thalassaemia to indicate whether there is any output of  $\delta$  and  $\beta$  chains from the affected chromosome.  $(\delta\beta)^0$  thalassaemia usually results from large deletions involving the  $\gamma\delta\beta$ -globin-gene cluster, which remove the  $\beta$ , $\delta$  genes but which leave either one or both of the  $\gamma$ -globin genes intact. As already mentioned they can be divided in to the $(\delta\beta)^0$ and  $(Ay\delta\beta)^0$  thalassemia, depending on the length of the deletion, that is, whether the Ay genes are involved or not. Carriers of thalassemia that were referred to the Primary Health Center were investigated. 5 ml of blood for molecular analysis and 2 ml of fresh blood for hematological lab was used. In this study, two families are investigated because of low MCV and MCH, normal HbA2 and high HbF. First, specific primers for  $\delta\beta$ -thalassemia were designed. DNA fragments were amplified by PCR. Known deletions causing  $\delta$   $\beta$ -thalassemia and HPFH were screened for in these families by gap-PCR method. DNA fragments were visualized on a 2%

agarose gel. The results showed that two families are carriers for the Asian-Indian inversion and deletion. Genotype-phenotype correlation was performed the same as globin gene server database.

#### P01.006

##### Genotyping of $\alpha$ -globin genes in Iranian $\alpha$ -thalassemia carriers

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##### Introduction:

Alpha thalassemia is more often caused by deletions involving one or both of the  $\alpha$ -globin genes.

A small number of point mutations, usually within the  $\alpha$ 2, have been characterized. The aim of

This study was molecular analysis of  $\alpha$ -globin genes in some Iranian people with low MCV, MCH and normal HbA2 and HbF.

##### Materials and Methods:

After obtaining informed consent, DNA was extracted from blood samples of 100 individuals referred from Primary Health Care (PHC) centers by salting out method. Multiplex Gap-PCR for common  $\alpha$ -globin gene deletions was performed. Then for individuals who did not have known deletions,  $\alpha$ 2 and  $\alpha$ 1-globin genes were sequenced by chain termination method.

The sequences were aligned against Z84721 accession number in GenBank and results were compared with globin gene server database.

##### Results:

Among 100 individuals, 47 individuals have had deletions in  $\alpha$ -globin gene including:

$-\alpha^{3.7}(27)$ ,  $-\alpha^{Med}(8)$ ,  $-\alpha^{4.2}(7)$ ,  $-\alpha^{20.5}(5)$ .

of the 53 remaining individuals who did not have known deletions in  $\alpha$ -globin genes, 23 samples had different mutations; including: PolyA<sub>4</sub>(8), PolyA<sub>6</sub> & 5nt(5), C.S(1), hemoglobin Adana, Cd28. The most common deletions and point mutation were  $-\alpha^{3.7}$  and PolyA, respectively.

Conclusion: Non deletion mutations can interact with each other or  $\alpha$ 0 thalassemia deletions to produce severe forms of HbH disease or even Hb hydrops fetalis. Thus screening for  $\alpha$ -thalassemia should be considered during genetics counseling of high risk couples of thalassemia for prenatal diagnosis. In addition,  $\alpha$ -thalassemia may alter the hematologic parameter in  $\beta$ -thalassemia carriers.

#### P01.007

##### A new polymorphism causes different restriction pattern by $\beta$ -Rsal in $\beta$ -globin cluster: application in PND

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The  $\beta$ -globin cluster is located at chromosome 11 and contains five functional genes. There are at least nine RFLP markers distributed all over the cluster. These markers are used routinely for carrier testing, prenatal diagnosis and haplotype analysis.

Working on the  $\beta$ -globin cluster haplotypes of some Iranian  $\beta$ -thalassemia carriers and their families, we observed a different pattern of digestion by Rsal restriction enzyme in many people. The aim of this study was to find out the cause of this pattern.

This study was performed on carriers of  $\beta$ -thalassemia and normal controls. After obtaining informed consent, DNA was extracted from peripheral blood. ARMS-PCR method was exploited for finding the common mutations in  $\beta$ -globin gene. PCR-RFLP was performed on  $\beta$ -Rsal polymorphic site. The primers and PCR conditions are from Weatherall & Clegg. DNA sequencing was performed on PCR products of  $\beta$ -Rsal marker by the same primers.

Some of the carriers of  $\beta$ -thalassemia and their parents had a different digestion pattern in  $\beta$ -Rsal polymorphic site. In the carrier people different mutations were found (IVSII-1, IVSI-110, IVSI-6, -88). When polymorphic restriction site of Rsal is absent in the sample, after digestion, the 1200 bp PCR product is cut to 694, 411 and 95 bp bands due to two constant restriction sites. In our cases the constant restriction site at position 411 has been changed (GTAC to GCAG) in heterozygous form. Hence, the restriction pattern by this enzyme creates 694 and 506 bp bands. This polymorphism is not associated to a specific mutation in  $\beta$ -globin gene and also was found in normal control people.

#### P01.008

##### Co-inheritance of Hemoglobin D and $\beta$ -Thalassemia Trait in three Iranian families

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$\beta$ -thalassemia is the most common genetic disorder in Iran, occurring more frequently in Northern and Southern areas. IVSII-1 and IVSI-5 are the most common mutations reported in the country. HbD, a hemoglobin variant occurs mainly in north-west India, Pakistan and Iran and differs structurally from normal HbA at 121 positions on  $\beta$  chain. Co-inheritance of HbD and thalassemia minor is not common and may alter the Hb electrophoresis pattern. Here we report three cases with combination of  $\beta$ -thalassemia and Hb D. None of them had symptoms of profound anemia and hematological indices were similar to the  $\beta$ -thalassemia heterozygote. Hb variant level in carriers was increased and no HbA was detected electrophoretically. After obtaining informed consent, the blood samples were collected in tubes containing EDTA. Genomic DNA was extracted using the salting out method. The mutation in  $\beta$ -globin gene was revealed by ARMS-PCR technique and confirmed by DNA sequencing. The region containing exon 3 was amplified for HbD and the PCR product of this amplicon was digested by EcoRI. The electrophoresis pattern suggested that all cases were homozygote for HbD. But, molecular analysis confirmed the presence of Cd 121 GAA>CAA in heterozygous form in combination with IVS II-I and IVS I-5. Hematological family study showed the mutations and HbD are in trans position. These mutations produce an unstable mRNA without any product and almost all of the globin output is from the chromosome carries HbD.

#### P01.009

##### Analysis of haplotypes associated with IVSI nt 130 of beta-globin gene reveals intriguing results

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Prevention of thalassemia is a national program. Premarital screening and prenatal diagnosis (PND) is in effect. For performing PND, we usually use direct mutation detection techniques like ARMS PCR. We routinely screen for more than 42 mutations identified in our center so far. If now mutation is detected we resort to direct DNA sequencing. We also use beta-globin gene linked RFLPs or SNPs for PND to increase the accuracy of PND. Sometimes haplotype analysis can be very helpful as well.

We have so far analyzed more than 6394 chromosomes from carriers of beta-thalassemia and in 16 cases the mutation was IVSI nt 130. This mutation is regarded as rare mutation in Iranian population. The families were mostly from two distinct geographical areas, namely Aghghola in Golestan province, west of Caspian Sea in the north of Iran (6 cases or 37%) and Meshkin shahr in Ardabil province, east of Caspian Sea, in north of Iran (5 cases or 31%). The other 4 cases were from Khozestan in South West and Gilan in North of Iran equally. Since populations in these two regions do not have much in common we decided to see if they share the same haplotype. We tested Hind III  $\psi\beta$ , Avall  $\beta$ , and Hinfl  $\beta$  RFLP sites for this purpose. All cases from Aghghola were + - + and all cases from Meshkin Shahr were - - + for these sites.

This shows that there were two different founder effects for this mutation.

#### P01.010

##### Non-Invasive prenatal diagnosis of $\beta$ -thalassaemia by SNP analysis using PNAs and Arrayed Primer Extension(APEX)

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The recent discovery of relatively abundant quantities of cell free fetal DNA in maternal plasma and serum has opened up new possibilities for the non-invasive prenatal diagnosis.  $\beta$ -thalassae mia is one of the most common autosomal recessive single-gene disorders in Cyprus.

In Cyprus, the mutation IVSI-110 accounts for 81% of all the cases. Therefore, the development of a non-invasive method for the Cyprus population is based on the detection of paternally inherited Single Nucleotide Polymorphisms (SNPs) as well as the direct detection of paternal beta-thal mutations.

Eleven SNPs with high degree of heterozygosity in the Cypriot population were selected and analyzed on 34 families and the informative SNPs were determined. In order to find a higher number of informative SNPs, the degree of heterozygosity in the Cypriot population was determined for 130 SNPs on 75 random samples using the Sequenom® MALDI-TOFF Mass Array genotyping analysis.

One of the approaches that are being developed is the Arrayed Primer Extension (APEX) method on the Genorama® Quattrolmager™ . We developed a DNA chip called "thalassochip" that contains 60 beta-thal mutations and 10 SNPs linked to the beta-globin locus. The APEX assay was applied on maternal plasma of 7 families using the informative SNPs; paternal allele of the fetus was non-invasively detected in 5 families.

Peptide Nucleic Acids (PNA) probes are used to suppress amplification of the maternal allele and unmask the fetal allele. The efficiency of PNA-mediated PCR clumping technique was tested on APEX and sequencing analysis.

#### P01.011

#### Delineation of deletions in beta globin gene cluster causing HPFH in Iran

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**Background:** Hereditary persistence of fetal hemoglobin (HPFH) and alpha thalassemia are heterogeneous disorders characterized by elevated levels of fetal hemoglobin (HbF) in adult life. The distinction between these conditions is subtle and is made on clinical and hematological grounds. Most HPFHs are caused by large deletions involving a variable extent of DNA segment on the beta-globin gene cluster. There are eight common forms of such deletions reported in different populations. In this study ten unrelated individuals with characteristic HPFH hematological profile were investigated to delineate their beta-globin gene cluster deletions.

**Aims and objectives:** Molecular analysis of 10 Iranian patients with low MCV and MCH, Normal HbA2 and high level of HbF (5%-15%) was carried out. They were referred from primary health care centers involving in the national prevention program for thalassemia.

**Materials and methods:** After obtaining informed consent, genomic DNA was extracted from peripheral blood. Multiplex gap PCR method was exploited for characterizing of 8 different deletions in beta-globin cluster causing delta-beta thalassemia or HPFH.

**Results and discussion:** Seven individuals from this group were shown to be heterozygous for the 13.4 kb Sicilian deletion, two were heterozygous for the Asian-Indian form of inversion-deletion Ggamma(Agama-delta-beta)<sup>0</sup> thalassemia and mutation for one of them was not identified. So far three types of deletional mutations in Iranian patients have been reported. These results confirm the previous findings.

#### P01.012

#### Reporting of Beta Thalassemia mutations frequency by DNA sequencing analysis in Iran

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Beta-thalassemia is the most prevalent genetic disorder in Iran. In order to control this autosomal recessive disorder several plans are in effect, including premarital genetic counseling and blood tests. Our unit has been chosen as National Reference Center for Prenatal Diagnosis. We are actively involved in prenatal diagnosis, carrier detection

and molecular analysis of mutations in beta-globin gene. We have started to perform DNA sequencing for all samples especially for unknown cases since July of 2005. We find that DNA sequencing is very suitable and informative for screening of mutations except deletions. DNA samples are usually tested for mutations like IVS-II-I, IVS-I-5, IVS-I-110, codon 5, codon 17, codon 41/42(-TTCT) and many common mutations and deletions. We have decided to use a comprehensive mutation screening approach and sequenced the gene by 3130 Genetic Analyzer. After detection of 2654 sequences in 2 years, we have found very different abundance for these mutations:-28tata box,-29tata box,-30 tata box,-88,-101,codon 2,codon15,codon36-37,codon39,codon82-83,codon121,IVS-I-5,IVS-I-110,IVS-II-1,IVS-II-666 and more than 35 other mutations.

#### P01.013

#### Molecular characterization of beta-thalassemia intermedia in Antalya population, Turkey

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Beta-thalassemia intermedia (Beta-TI) is a term used to define a group of patients with beta-thalassemia in whom the clinical severity of the disease is somewhere between the mild symptoms of the beta-thalassemia trait and the severe manifestations of beta-thalassemia major. Beta-TI shows both clinic and genetic heterogeneity. The purpose of this study was to analyze the relation between the genotype and phenotype in Turkish patients with beta-TI living in Antalya, Turkey. A total of 32 patients with beta-TI were evaluated for mutations and their clinical findings. Eight different mutations [-30 (T-A), Cod 3 (+T), Cod 8(-AA), Cod 39 (C-T), IVS1.6 (T-C), IVS1.110 (G-A), IVS2.1 (G-A), IVS2.745 (C-G)] were found in our study. The IVS1.6 (T-C) was the commonest beta-mutation, occurring in both homozygous state in five patients and compound heterozygous state in eight patients. The IVS2.1 (G-A) was the second beta-mutation in eight patients as homozygous state in two patients. In a family with beta-thalassemia, two sibs were compound heterozygote for IVS2.1(G-A) and IVS1.110 (G-A). One of them was female with beta-TI, while other was male with beta-thalassemia major. In addition, our findings were compared with literature and the mutation profile in beta-TI patients was differently found in our population than others. In conclusion, our data suggest that modifier genes should be screened together with beta-globin gene mutations in patients with beta-thalassemia intermedia to give correct genetic counseling and to provide the effective treatment.

#### P01.014

#### Effect of alpha-gene numbers and XmnI Polymorphism on the phenotype of HbE/beta thalassemia patients

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**Introduction:** HbE [β26 (B8) Glu-Lys] with β thalassemia (HbE/β thalassemia) results in a clinically severe condition. HbE/β thalassemia has a very variable clinical phenotype. Some of the possible explanations for the observed variable clinical severity are coinheritance of α-thalassemia and XmnI polymorphism. **Objective:** To determine the frequency of XmnI polymorphism, α deletion and triplication in HbE/β thalassemia patients and to study their effect on the phenotype of Patients. **Material and Methods:** Subjects were 85 HbE/β thalassemia patients. Patients were divided into three subgroups according to a scoring system based on seven clinical criteria as mild (score 0-3.5), moderate (score 4-7) and severe (score 7.5-10). α deletions and XmnI polymorphism were studied by GAP-PCR and PCR-RFLP respectively. **Results:** α deletion was found in 18 (21.2%) out of these 18 patients 12(11 αα/-α<sup>3.7</sup> & 1 αα/-SA) were from Gp1 and 6(αα/-α<sup>3.7</sup>) were from Gp2. α triplication was found in 7(8.2%) out of these 7 patients 5 were (αα/ααα<sup>anti-3.7</sup>) from Gp3 and 2 were (αα/ααα<sup>anti-3.7</sup>) from Gp2. XmnI was found in 53(62.3%), out of which 43 were heterozygous(+/−) & 10 were homozygous(+/+). XmnI +/+ was present in 6 Gp1 & 4 Gp2 patients, while XmnI −/− was present in 8 Gp1, 15 Gp2 & 20 Gp3 patients. **Conclusion:** Patients with coexisting α deletion, required lesser transfusions and had less severe phenotype while patients with α triplication were on frequent transfusions and had severe phenotype. XmnI poly-

morphism in homozygous state was observed to alleviate the severity while in heterozygous state it had no effect on the disease severity.

#### P01.015

#### Delineation of deletions causing δβ thalassemia and HPFH in Iran

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<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>Molecular Medicine Dept., Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Tehran, Islamic Republic of Iran, <sup>4</sup>Ahwaz Medical sciences university, Ahwaz, Islamic Republic of Iran, <sup>5</sup>Childrener Medical center, Tehran university of Medical Sciences, Tehran, Islamic Republic of Iran. Hereditary persistence of fetal hemoglobin (HPFH) and δβ thalassemia are heterogeneous disorders characterized by elevated levels of fetal hemoglobin (HbF) in adult life. The distinction between these conditions is subtle and is made on clinical and hematological grounds. Most HPFs are caused by large deletions involving a variable extent of DNA segment on the β-globin gene cluster. There are eight common forms of such deletions reported in different populations. In this study ten unrelated individuals with characteristic HPFH hematological profile were investigated to delineate their β-globin gene cluster deletions.

**Aims and objectives:** Molecular analysis of 10 individuals with low MCV and MCH, Normal HbA2 and high level of HbF (5%-15%) was carried out. They were referred from primary health care centers involving in the national prevention program for thalassemia.

**Materials and methods:** After obtaining informed consent, genomic DNA was extracted from peripheral blood. Multiplex gap PCR method was exploited for characterizing of 8 different deletions in β-globin cluster causing δβ thalassemia or HPFH.

**Results and discussion:** Seven individuals from this group were shown to be heterozygous for the 13.4 kb Sicilian deletion, two were heterozygous for the Asian-Indian form of inversion-deletion Gy(Ayδβ)<sup>0</sup> thalassemia and mutation for one of them was not identified. So far three types of deletional mutations in Iranian patients have been reported. These results confirm the previous findings.

#### P01.016

#### Detection of the most prevalent deletional and non deletional mutation among Iranian carriers of Alpha-Thalassemia

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**Background:** A variety of deletions and point mutations have been described which decrease α globin gene expression. Most affected fetuses do not survive till birth or die shortly thereafter.

**Objectives:** The exact determination of gene defect for thalassemia carriers is essential for premarital screening. It will also help the genetic counselor to advise the family accordingly.

**Methods:** One hundred and thirty subjects having referred to us. Multiplex Gap PCR, And some molecular methods was carried out to detect any existing deletional or nondeletional mutation in their alpha globin genes.

**Results:** The following genotypes were identified:

{-α<sup>4.2</sup>/αα }, {- α<sup>3.7</sup>/- α<sup>3.7</sup>},{-α<sup>3.7</sup>/-α<sup>4.2</sup>}

Poly A signal mutation (AATAAA > AATAAG )

Termination codon Mutation ( TAA > CAA ) Or: -5nt deletion ;

(α<sup>3.7</sup> / - α<sup>20.5</sup>) & (-Med / α α)

Frequencies of these mutations was determined which revealed the 3.7 Kb deletion as the predominant mutation among alpha-thalassemia. carriers in this study. The frequency of all other types were less than 35%.

**Conclusions:** Among nondeletional mutation, α<sup>5nt</sup>, was the most frequent allele in our study population (9.25%) Followed by α<sup>PA-2(4A>G)</sup> (4.12%). In regard to cd19 that has a 12.2% prevalence in south of Iran(Harteveld, et al,2003) it seems that this mutation is not dominant in our studied population.

Dominant mutations in poly A signal in our study was α<sup>PA-2 (4A>G)</sup> which has a replacement in nucleotide 4 A>G.

#### P01.017

#### Molecular analysis of thalassemia intermedia in Iran

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**Background:** β-thalassemia is an autosomal recessive disorder caused by more than 200 different mutations in gene coding for β-globin (HBB) of the hemoglobin tetramer Thalassemia intermedia is a clinical definition applied to patients whose clinical phenotype is milder than thalassemia major.

**Aims and objectives:** Molecular analysis on α- and β-globin genes mutations in 49 Iranian thalassemia intermedia patients.

**Materials and Methods:** After obtaining informed consent the patients were included in the study. The genomic DNA was extracted from peripheral blood using standard salting out method. Allele-specific polymerase chain reaction was performed for common β-thalassemia alleles and then direct β-globin gene sequencing amplification was performed. β-globin gene haplotypes were constructed from eight restriction fragment length polymorphism (RFLPs) in the β-globin cluster and also -158GgXmnI (C to T). Screening of common α-globin gene deletions and triplication was performed by Gap-PCR.

**Results and discussion:** Among 49 thalassemia intermedia patients, 17 had IVSII-1 (G to A) mutation in homozygous form and 10 in compound heterozygous with other mutations. -α<sup>3.7</sup> and -MED gene deletions were found in heterozygous form in two and one of the above cases, respectively. In five cases only one mutation was found in β-globin gene and in these patients one had α-globin gene triplication. Analysis of polymorphic markers in patients with IVSII-1 mutation showed haplotype III and allele T in XmnI marker.

These data show the heterogeneity of molecular basis of thalassemia intermedia in Iran and suggest the role of modifier genes other than α-globin gene determinants.

#### P01.018

#### A novel frameshift mutation (-G) at codon 24 of the beta-globin gene in an Iranian woman

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Thalassemia is an inherited disorder characterized by an imbalance in the synthesis of α- or β-globin chains. This leads to a decreased hemoglobin synthesis and causes a hypochromic microcytic anemia.

Since 1997, in Iran, every couple that wants to marry is referred to one of the Primary Health Centers (PHC) by the marriage registry offices. These couple to be are tested for thalassemia by doing cell blood count (CBC0 and A2 level measurement. Eventually they may be sent to one of several medical genetics centers dedicated for prenatal diagnosis of thalassemia.

Individuals with low MCV (<80 fl) and MCH (<27 pg) and high Hb A2(>3.5%) level are referred for further investigation which may include prenatal diagnosis.

During investigating one of these couples with low MCV and MCH and raised A2 were investigated by molecular methods. A previously undescribed mutation causing a frameshift [ (-G) Codon 24] in the beta-globin gene was identified in a 23 years old Iranian woman . The hematological data for this lady was (MCV=63.0, MCH=19.0, A2=5.9 , F=0.2). Her DNA was tested for 19 common beta-globin gene mutations. No mutation was detected. Direct DNA sequencing showed absence of a G nucleotide in Codon 24 on both strands. We looked up data bases and available references for this mutation. No reported case was seen. We believe that this is a novel mutation causing a frameshift. We are in the process of investigating the frequency of this mutation by designing ARMS/PCR primers.

#### P01.019

#### Molecular analysis of two families with Hb Lepore

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Thalassemia syndromes are among hemoglobinopathy disorders inherited as autosomal recessive trait, caused by mutation in β-globin

gene. Most of these mutations are point mutation and large deletions are not common. A considerable number of deletions of variable size and position that involve the β-globin gene cluster on chromosome 11 are associated with the clinical entities of δβ thalassemia.

δβ-thalassemia normally results from deletion involving either δ- and β-globin genes or the Ay-, δ- and β-globin genes.

In this study six individual from two families were investigated because of low MCV and MCH, high HbF and normal HbA2 referred from primary health care (PHC) centers to our lab for further investigations.

PCR amplification was performed for known deletions causing δβ-thalassemia and HPFH by gap-PCR methods.

In Hb electrophoresis an extra band was appeared. Molecular analysis showed that the two affected individuals from one of the families are homozygous for Hb Lepore and the remaining four cases carry Hb Lepore in heterozygous form. One of the affected cases was transfusion dependent. Genotype Phenotype correlation was compatible with cases presented in globin gene server database. Hb Lepore usually causes mild anemia with microcytosis and hypochromia in the heterozygote (beta+thal). The molecular basis of this Hb variant is a (aprox) 7 kb deletion from the distal part of δ-globin gene to proximal region of β-globin gene.

#### P01.020

##### Hb F Malta I in association with Hb F Sardegna and Hb Valletta; triple heterozygosity at the human Gy, Ay and β globin genes suggest interplay between flanking regulatory sequences in the developmental control of globin gene switching

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Here we document for the first time data on unique families from Malta in whom heterozygosities at the Gy, Ay, and β globin genes have segregated among families to produce probands that were heterozygotes at the three major non-α genes, such that the six globin products could be separated and quantified. 136 newborn were found with Hb F Malta I on isoelectricfocusing. Further testing by reverse phase LC showed heterozygosities at the β globin gene ( $\beta$ A /  $\beta$ <sup>Valletta</sup>) and the Ay globin gene (AyI / AyT) confirmed by DNA sequencing in 8. The probands were genotyped at the Xmn I site in the 5' Gy promoter that is known to be associated with increased γ globin gene output in anaemic adults and the (AT)<sub>x</sub>T<sub>y</sub> polymorphism in the 5' β globin gene region known to down-regulate β globin gene expression subject to BP1 binding. Seven were Xmn I negative and (AT)<sub>7</sub>T<sub>7</sub> and with [Gy<sup>FMaltaI</sup>+ AyI] / [Gy0 + AyT] = 0.90 that was significantly different from the other triple heterozygote with Xmn I negative and (AT)<sub>9</sub>T<sub>5</sub> and [Gy<sup>FMaltaI</sup>+ AyI] / [Gy0 + AyT] = 0.80 ( $p < 0.037$ ). The data suggested interplay between the Xmn I and the (AT)<sub>x</sub>T<sub>y</sub> sites around a fulcrum of the Y / PYR sequences close to the pseudo-β sequences and that acted to control globin gene expression differentially between neonates and adults.

#### P01.021

##### A case of Hb Torino in an Italian family

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Alfa thalassemias are haematologic diseases arising from more than 80 different genetic alterations, affecting one or both copies of the duplicated α globin genes (α1 and α2) located in 16p13.3.

Although most causative alterations are large genomic deletions, at least 48 non-deletional point mutations have also been reported so far.

We report here a case of Hb Torino found in an Italian family: the proband is a young boy aged six, who presented haematological parameters similar to α thalassemia.

The α-globin2-specific PCR product were amplified. Direct sequencing of amplified PCR product showed the presence of Hb Torino (Cod43 TTC->GTC, Phe->Val) in both chromosomes in the proband. Since Hb Torino was detected in the homozygous state and it is quite a rare variant we decided to extend the analysis to patient's parents.

The father resulted to be an heterozygous carrier for Hb Torino, while the mother had a 3.7 deletion in the heterozygous state. Then we concluded that proband had the Hb Torino in one chromosome and a 3.7

deletion in the other chromosome.

The correct diagnosis, improved after the case history, included the presence of the base substitution causing Hb Torino and the α 3.7 deletion, both in the heterozygous state.

Our data underline that the molecular screening of α thalassemia, associated to the family study are useful to better characterize the genotypes involved and perform an appropriate genetic counselling.

#### P01.022

##### Hemoglobin Lepore chromosome in Serbia: a report of a novel Lepore haplotype

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Hemoglobin (Hb) Lepore is a thalassemic hemoglobin variant characterized by normal alpha-globin and fused delta/beta-globin chains. Heterozygosity for this abnormality resembles a beta-thalassemia trait, while homozygotes have a severe form of beta-thalassemia. Hb Lepore-Boston Washington (BW) is the most common type of Hb Lepore. The chromosomal background heterogeneity has been assessed in Hb Lepore BW chromosomes, suggesting its multicentric origin. Molecular characterization of Serbian patients with thalassemia syndromes in last ten years revealed that Hb Lepore is the most common cause of thalassemia phenotype in the population of Serbia (25%). Three thalassemia major patients (compound heterozygotes for Hb Lepore and beta-thalassemia mutation) and 36 heterozygous Hb Lepore carriers were characterized in 15 unrelated families. Molecular detection of Hb Lepore gen was carried out by gap-PCR analysis. Sequencing analysis showed that all Hb Lepore genes were of BW type. Moreover, they were all associated with the same intragenic beta-globin gene polymorphisms, framework 2. Additionally, we have studied beta-globin gene cluster haplotypes and their association with Hb Lepore gene in Serbian population by PCR-RFLP analysis of 8 polymorphic sites (Hinc II/epsilon, Xmn I/5'Ggamma, Hind III/Ggamma, Hind III/Agamma, Hinc II/pseudobeta, Hinc II/3'pseudobeta, Ava II/beta, BamHI/3'beta). Haplotype analysis revealed a novel haplotype associated with Hb Lepore BW gene (+---+-). The same haplotype was found in healthy individuals of Serbian descent. The high frequency of Hb Lepore BW hemoglobin variant in Serbian population, the homogeneity of Hb Lepore BW haplotype, as well as its uniqueness, suggest that it most probably originated in Serbia.

#### P01.023

##### Control of Thalassemia in Iran, a National Success Story

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Thalassemia is the most prevalent single gene disorder in Iran and most part of the world. Now more than 18000 patients live in Iran.

Prenatal diagnosis of thalassemia started, in Iran, as early as 1991 by sending samples abroad and as early as 1994 it became feasible to do it in Iran. National Program for Prevention of Thalassemia has been started in 1997 and the religious FATWA was given in 1996 to allow prenatal diagnosis (PND). From 1997 every couple who wants to get married is tested for being a carrier of thalassemia. If both partners are carriers or are in doubt of their carrier status are referred to one of several prenatal diagnosis centers throughout the country. Regular visits and inspections are carried out to ensure the best performance. Every PND done is reported to the Genetics Office at CDC.

There are more than 10 medical genetics labs in Iran and most of them active in doing PND for thalassemia. Most of these laboratories have been organized as being a network and families are referred to one of these labs via the Health Centers throughout country.

In our medical genetics lab at Kawsar Genomics and Biotechnology Complex we have performed more than 2000 PNDs. We have also analyzed more than 4000 samples referred to us for thalassemia. Only one mistake has been made out of 2000 PNDs which may indicate application of best QA and QC.

**P01.024****Molecular mechanisms underlying thalassemia intermedia in Iran**

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To improve the differentiation of thalassemia intermedia from other hemoglobinopathies in Iran, four known genetic mechanisms, including *Xmn1*  $\delta\gamma$  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 *Xmn1*  $\delta\gamma$  polymorphism, the Sicilian  $\delta\beta$  deletion and four  $\alpha$ -globin mutations ( $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $-\text{MED}$ ,  $\alpha\alpha^{\text{anti-}3.7}$ ) were studied using PCR-based techniques. The inheritance of the *Xmn1*  $\delta\gamma$  polymorphism with severe  $\beta$ -thalassemia alleles in the homozygous or compound heterozygous state was the predominant mechanism observed in 56.5% of individuals. In 8.7% of cases, this status overlapped with the  $-\alpha^{3.7}/\alpha\alpha$  genotype. The second most frequent cause for thalassemia intermedia (15.2%) was the inheritance of mild  $\beta$ -thalassemia alleles, including IVS-I-6 (T>C), -88 (C>A) and +113 (A>G). In 4.3% of subjects the Sicilian  $\delta\beta$  deletion was identified. HbS in association with  $\beta^0$ -thalassemia was found in 2.2% of patients, who had been misdiagnosed as thalassemia intermedia. In 21.7% of cases no causative genetic alteration could be identified. Our results reflect the diversity underlying thalassemia intermedia in Iran, and the limitations of the applied clinical, hematological and molecular approaches for correct diagnosis. Some of our unresolved cases will offer an opportunity to discover additional molecular mechanisms leading to thalassemia intermedia.

**P01.025****Prenatal diagnosis of cystic fibrosis: the 18-year experience of Brittany (western France)**

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**Objective:** This study reports 18 years of experience in prenatal diagnosis (PD) of cystic fibrosis (CF) in a region where CF is frequent and the uptake of PD common (Brittany, western France). **Method:** All PDs made over the period 1989-2006 in women living in Brittany were collected. **Results:** We recorded 268 PDs made in 1 in 4 risk couples, plus 22 PDs directly made following the sonographic finding of echogenic bowel. Most of the 268 PDs were done in couples already having CF child(ren) (n=195, 72.8%). Close to one fifth followed cascade screening (n=49, 18.3%), which identified 26 new 1 in 4 risk couples among the relatives of CF patients or of carriers identified through newborn screening. The remaining PDs were mainly made in couples whose 1 in 4 risk was evidenced following the diagnosis of echogenic bowel in a previous pregnancy (n=22, 8.2%). Although patients' life expectancy has considerably improved, in our population the great majority of couples chose pregnancy termination when PD indicated that the fetus had CF (95.9%). **Conclusion:** This study describes the distribution of PDs according to the context in which the 1 in 4 risk was discovered and highlights the real decisions of couples as regards pregnancy termination after a positive PD.

**P01.026****Strategy for prenatal diagnosis of cystic fibrosis in Serbia**

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Cystic fibrosis is the most common autosomal recessive disease in Caucasians, caused by more than 1500 mutations in CFTR gene. In Serbian CF patients 21 different CF mutations were found accounting for 82% of CF alleles.

Since 1996, we have performed 102 prenatal diagnoses for 76 couples with 11 different genotypes. One case included twin pregnancy and another a CF affected mother. In one family-at-risk parent was a carrier of complex allele (two mutations in *cis*).

Total of 63 families were fully informative for direct DNA analysis, which was performed using heteroduplex analysis (PAGE), ARMS-PCR (Elucigene™CF 29 kit, Orchid) and DGGE analysis of PCR amplified exons 1-24. In other 13 families, only one parental mutation was known, so prenatal analysis was done by indirect DNA analysis (haplotype analysis for 6 diallelic sites and one tetranucleotide repeat). Materials used for fetal DNA analysis were mostly CVS samples (68 cases), amniocytes in 28 cases and fetal blood in 6 cases. Results showed that 19 fetuses were affected, 50 were carriers and 33 fetuses were healthy.

Since 2006, we have started screening for the presence of CFTR mutations in couples with echogenic bowel detected on ultrasound investigation during the second trimester of pregnancy. From 21 cases, in 16 only the couple was tested, and in 3 cases both parents and fetus were tested. No positive cases were found.

If the causative mutations were identified before pregnancy, results were given within few days, indicating that the current strategy using a combination of methods mentioned above provide rapid and reliable prenatal diagnosis for all families at risk in our country.

**P01.027****CFTR haplotypes associated with p.S466X mutation among Iranian CF patients**

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Cystic fibrosis (CF) is the most common inherited disorder in Caucasian populations, with over 1400 mutations identified in the Cystic Fibrosis Transmembrane conductance Regulator (CFTR) gene. We have screened 69 Iranian CF patients, and 1 CBAVD patient for mutations and polymorphic sites in the complete coding region, and its exon/intron junctions, of their CFTR genes, using different methods, such as ARMS (amplification refractory mutation system)-PCR, SSCP (single stranded conformation polymorphism) analysis, restriction enzyme digestion analysis, direct sequencing, and MLPA (Multiplex Ligation mediated Probe Amplification).

Based on our work, the third most prevalent mutation in Iran was p.S466X. It was found in 5.7 % (8/140) of the CFTR genes from Iranian CF patients, and was only observed in homozygous state. These 4 patients were from Tehran, Khorasan, Hamadan and Markazi provinces. The parents of these patients all had consanguine marriages, however since that the mutation was present in families from different regions; it seems to be a relative frequent mutation in Iran. This mutation is rare worldwide, but has a frequency of 0.5% in Serbia and Montenegro. Interestingly, the patients with p.S466X mutation were homozygous at some of intragenic polymorphic sites and showed similar haplotypes. These were: IVS8 TGm and Tn (TG12-T7\_\_TG12-T7), IVS6a (TTGA6/TTGA6), intron 9 nt1525-61 (G/G) and exon 10 nt1540 (A/A; M470/M470). The polymorphisms were seen in direct sequencing.

**P01.028****Identification and characterization of three CFTR gene partial duplications**

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**Background:** Cystic fibrosis and disorders related to Cystic Fibrosis transmembrane Conductance Regulator (CFTR) pathology are mainly due to point mutations scattered over the whole CFTR gene. Search for large CFTR rearrangements using semi-quantitative fluorescent multiplex (QFM) PCR assays is now part of the molecular diagnosis and allows to identify 2% of CF alleles. Rearrangements mainly comprise single or multiple exon deletions; duplications are rare and

are indeed more difficult to detect and characterize. Of the four *CFTR* duplications reported, three were detected in our laboratory by QF-PCR, in two CF patients and a CBAVD patient. They involved exons 4-8, 10-18 and 11-13, respectively, in trans of another *CFTR* mutation. Methods: The duplications were characterized by using a combination of long-range (LR) PCR, digestion of LR-PCR products and sequencing. Results: Two duplications were fully characterized, in direct tandem each: dup10\_18 ( $\approx$ 70kb long), and dup11\_13 ( $\approx$ 17kb long). Characterization of the remaining dup4\_8 is in process. However, given the classical CF phenotype of the patient, we hypothesize that the duplicated region is located inside the *CFTR* gene and interferes with the transcription, translation or maturation process, thus resulting in a null mutation. Conclusion: Effective tools are required to detect duplications, which may indeed be under-diagnosed. Refinement of the breakpoints is important to confirm a deleterious effect and should contribute to understand the duplication mechanism.

#### P01.029

#### A French collaborative study indicative of a very low classical-CF penetrance of R117H; implications for genetic counselling.

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Background: The R117H-associated phenotypes vary from classical CF to no clinical disease and have made genetic counselling difficult. Since implementation of CF NBS, the observed high R117H frequency among neonates with elevated IRT and two mutations has reinforced this issue. Methods: Two retrospective studies were conducted: 1) a phenotypic study on 263 patients with two *CFTR* mutations including at least one R117H; 2) a retrospective 2002-2005 epidemiological study, aimed to determine the frequency of R117H and other frequent mutations in about 6000 healthy individuals without family history of CFTR pathology. Results: 1) Among the 263 patients, including 92 neonates, detailed clinical features were available for 247: severe classical CF, n= 2; isolated CBAVD, n= 60; other *CFTR*-related disorders (CFTR-RD), n= 109; healthy, n= 76 (65 neonates, reduced follow up period); 2) Based on R117H and F508del allelic frequencies in the general population of 0.25% and 1.0%, respectively, the [F508del]+[R117H] genotype prevalence was evaluated at 1/20,000, the CFTR-RD penetrance at 4.2% and the CF penetrance at 0.06%. Conclusion: The very low penetrance of R117H with regard to classical CF leads to consider R117H no longer as a CF-causing mutation and to reassure patients and their families in view of genetic counselling.

#### P01.030

#### The molecular genetic study *CFTR* gene in the group of Russia CF-patients

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Cystic fibrosis (CF) is a common and generally severe autosomal recessive disorder in the European population, caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) on chromosome 7q31.

In the Russian population, cystic fibrosis is characterized by the presence of two prevalent mutations, the F508del and CFTRdele2,3, which account for ~55% and ~6% of the CF alleles, respectively.

This study includes 120 unrelated Russian CF patients affected by a classical form of cystic fibrosis.

Using a comprehensive *CFTR* gene analysis protocol ( $\Delta$ F508,  $\Delta$ I507,

1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, L138ins, G542X, W1282X, N1303K, 3849+1C-T, R334W, CFTRdele2,3) mutations have been previously identified in 112/120 (93,3%) patients including 76 (63,3%) with two revealed mutations and 36 (30%) with one revealed mutation. The current study present the result of investigations by using a commercial kit (CF OLA assay, Abbot, Rungis-France) in the 44 remaining samples with only one or without revealed *CFTR* mutation. Three *CFTR* mutations at four patients have been in addition identified. Mutation 2789+5g>a was identified in two patients (0,8%). Two mutations were identified in one patient each (0,4%): R1162X, 3120+1g>a. Mutations identified by using a commercial kit in addition in 4 (1,6%) of the 240 *CFTR* chromosomes in investigated group.

#### P01.031

#### Identification of novel mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene in the Greek population

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Cystic Fibrosis is the most common autosomal recessive disorder, with 5% carrier rate in the Greek population. The Greek population has one of the highest rates of *CFTR* mutation heterogeneity.

In this study we report 16 novel variants in the *CFTR* gene identified by DGGE analysis and direct sequencing. Of these five were synonymous variants, 7 missense mutations, 2 frameshift mutations leading to premature termination codon and two intronic substitutions. The effects of these mutations were assessed in combination with the clinical phenotype and using *in silico* analysis. The missense mutations were assessed using "PolyPhen" and "SIFT". The impact of the silent mutations and the intronic substitutions on splicing elements was analysed using "SSF: Splicing Sequences Finder". Majority of findings included changes in splicing factor binding.

Case	Novel Mutation/ variant	Other <i>CFTR</i> mutation/ variant	Clinical Phenotype	Polyphen	SIFT
1	c.538_539insAC (L136H-fs153X)	F508del	111.5 mEq/L; Classical CF; 3 mos		
2	c.3946_3947delTG (V1272fs1300X)	F508del	118.6mEq/L; Classical CF ; 2 yrs		
3	F319V	TG11T5/ TG11T7	Fetal Echogenic bowel; meconium ileus; 59.5 mEq/L	possibly damaging	T 0.48
4	L541P	N1303K	90meq/L; azoospermia; 38yrs	Probably Damaging	NT 0.00
5	L1227L	P936T	Failure to Thrive; 6yrs		
6	P936T		Oligospermia	Probably Damaging	T 0.38
7	R1158R	T966I	Oligoasthenospermia		
8	D1275D		50mEq/L; Malabsorption Syndrome; 5 yrs		
9	2622+3A>G*		Oligospermia		
10	V1212F		GP	possibly damaging	T 0.42
11	F305V		GP	benign	T 0.33
12	2752-18delT*		GP		
13	S511C		Azoospermia	benign	T 0.06
14	L1414S		GP	possibly damaging	T 0.06
15	L1408L		Bronchiectasis; 30yrs		
16	L346L		Pancreatic Dysfunction; 7yrs		

\* possible splicing mutation;

GP: General Population; NT: not tolerated; T: Tolerated

#### P01.032

#### Molecular-genetic and clinical analysis of cystic fibrosis in Republic of Moldova

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Objective: We intended to study relations between genetic and clinic polymorphism in patients with cystic fibrosis (CF).

**Methods:** Were investigated 78 patients with CF aged from 2 months to 2 years, 46 boys and 32 girls by mean of polymerase chain reaction (PCR).

**Results:** In 64,1% were detected mutations - delTAF508 - in 57,7% (15,4% homozygous and 42,3% heterozygous), R334W - heterozygous - 3,8%, N1303K - heterozygous - 1,3%, MetH polymorphism - homozygous - 1,3%. In all homozygous and in 88% of heterozygous of delTAF508 was mentioned pancreatic insufficiency and extremely severe injury of lungs. In 94,3% of patients the process starts before one year. From 8 died patients 75% were homozygous for delTAF508. In 35% of patients with this mutation was revealed Ps. Aeruginosa, in 40% of patients was hypotrophy. R334W characterized by slow progression of broncho-pulmonary injury and absence of pancreatic insufficiency. In homozygous of MetH polymorphism the disease was slowly progressive, despite early start in 3 months. 12 patients (15,4%) were older than 18 years. In half of them were delTAF508 mutation (heterozygous), in 8,3% - homozygous. In 16,7% mutations were not identified.

**Conclusion.** The clinical pattern and prognosis of CF depends from type of mutations. The frequency of delTAF508 in Moldova is 57,7%. In 64,1% of cases mutations are identifiable. In 47,7% patients was determined only one mutation from compound with delTAF508, R334W, N1303K. It diminishes the possibilities of prenatal diagnosis and lead to necessity to wide range of major mutations of causal gene.

#### P01.033

#### Association of polymorphism in the endothelial nitric oxide syntase gene and clinical features in Russian CF patients homozygous for F508del mutation

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The association of 27 b.p.VNTR polymorphism in 4 intron of eNOS gene and the clinical features of cystic fibrosis was investigated in 101 Russian CF patients homozygous for F508del mutation. All patients were subdivided for groups according to their eNOS genotype, the first group - A/A and A/B genotypes (33 patients); the second group - B/B genotypes (68 patients). The age of onset of lung and intestinal disease symptoms, the age of diagnosis, severity of disease progression, FVC index, height-weight indexes, colonization by *S. aureus* and *P. aeruginosa* and other microorganisms, hepatobiliary disease, meconium ileus and distal intestinal obstructive syndrome in anamneses were evaluated. FVC index was significant lower in patients with A/A and A/B eNOS genotypes ( $69,37 \pm 4,09\%$ ) than patients with B/B genotype ( $80,57 \pm 3,31\%$ ;  $p=0,032$ ). Liver cirrhosis was more frequent among patients with B/B eNOS genotype (22,1%) than in patients with A/A and A/B eNOS genotypes (6,1%;  $p<0,05$ ). No other associations were revealed.

#### P01.034

#### MBL influence cystic fibrosis associated liver disease in children?

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**Background:** Genetic modifiers like mannose binding lectine(MBL2) gene influence occurrence and severity of CF associated liver disease(CFLD). Variants of MBL genotypes encode low serum levels of MBL protein. Aim study was to asses the MBL serum levels in CFLD patients (pts), comparing to CF pts without liver disease. **Methods:** Study was performed in Pediatric II Department and National Cystic Fibrosis Centre Romania. 35 CF children aged 2-18 years, presented to annual assessment were evaluated. We consider 3 groups of patients, as follows: group 1(21 CFLD patients), group 2(11 pts CF no CFLD), group 3(15 controls). Groups were age matched. Patients associating diseases who influence MBL level were excluded. MBL assay procedure was performed using MBL Oligomer ELISA kit. Data were statistically analyzed with ANOVA. **Results:** Among CFLD pts (group 1) MBL average was half of control's average ( $p=0,001$ ). Group 2 had an average lower with 13% comparing to controls. 46% CFLD pts were MBL deficient. **Conclusions:** Lower MBL average in CFLD patients sustains that MBL deficiency is associated factor for CFLD.

Increased MBL levels in CFLD patients could be resultant of increase growth hormone levels in childhood or predominance of "wild" MBL2 genotype in our patients. MBL2 genotyping is necessary to identify the patients predisposed to develop CFLD.

#### P01.035

#### CFTR and ENaC genes study in African patients with cystic fibrosis-like disease

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The defect in chloride and sodium transport in cystic fibrosis (CF) patients is a consequence of loss of functional interaction between cystic fibrosis transmembrane conductance regulator (CFTR) and the epithelial sodium channel (ENaC).

To study patients with CF-like symptoms and to relate the disease to gene mutations of both CFTR and ENaC genes, we collected clinical data and DNA samples from 60 African patients with CF phenotype. The CFTR gene was first analysed for mutations in all patients by DH-PLC followed by direct sequencing, whereas the SCNN1A, SCNN1B and SCNN1G subunits of ENaC gene were analysed by sequencing in all patients who carried only one CF mutation. The frequency of all identified ENaC variants was established in control group of 200 health individuals.

In total, four different CFTR mutations, including one previously undescribed missense mutation (p.A204T) were identified in five patients. In addition, ENaC gene sequencing in these 5 patients detected 8 ENaC variants, (c.72T/C and p.V573I) in SCNN1A, (p.V348M, p.G442V, c.1473+28C/T, and p.T577T) in SCNN1B, and (p.S212S, c.1176+30G/C) in SCNN1G. The missense mutation (p.V348M) was not found in the control group. We could also not find any p.T577T silent polymorphism nor any c.1176+30G/C intronic nucleotide change.

Our data thus show that an exploration of African patients with CF-like symptoms is mandatory and should include ENaC gene sequencing in absence of identified two CFTR causing mutations. The combination of mutations in both genes could provide a genetic explanation for an involvement in disease.

#### P01.036

#### Detection of CFTR gene rearrangements in Spanish population

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Over 1,500 mutations have been reported in the CFTR gene. We have identified more than 200 CF mutations in Spanish families ( $n=1,020$ ) accounting for 97% of CF alleles. The CFTR50kbdel mutation is a complex rearrangement involving exons 4-7 and 11-18. This deletion is relatively common in Spanish population (0.3%). In order to provide a faster detection than Southern blot we have applied quantitative real-time PCR analysis to assess this deletion in 11 uncharacterized alleles from patients fulfilling CF criteria diagnosis. We have designed primers (Primer express 3.0 software) for 10 exons, 8 concerning the flanking regions (3, 4, 7, 8, 10, 11, 18, 19) and two others into the deleted regions (6a, 17a). Genomic DNA samples were scanned for each CFTR region as well as for the b2-microglobulin, as endogenous gene, using Power SYBR Green PCR Master Mix in a quantitative real-time PCR (ABI 7300). Positive CF controls and wild type samples were included in the study. Comparative analysis of normalized  $C_t$  ( $2^{-\Delta\Delta C_t}$ ) from patients and controls permitted us to estimate the dosage. We have detected dosage changes in four samples out of 11 analyzed. The CFTR50kbdel was detected in two patients. Another deletion and one insertion were also identified.

We conclude that this technique shows a high sensitivity and is suitable to identify CFTR large rearrangements, deletions and insertions, permitting us to improve the detection rate in Spanish CF patients.

**P01.037****Validating assays for relative quantification of CFTR cDNA from nasal epithelial brushing**

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Real-time PCR has proven to be a useful method to quantify gene expression in samples with small number of cells. We have applied this technology to validate the relative quantification of CFTR RNA from nasal brushing of CF patients in a Taqman assay (ABI 7300). Four endogenous genes have been evaluated (HPRT1, beta2-M, GUSB, PMCA4). Standard curves were performed for CFTR and endogenous genes from the cell line HEK293 over expressing CFTR, nasal polyp and nasal epithelial (NE) samples. The selection of suitable endogenous genes for normalization is a prerequisite for accurate determination of expression level. Hence, PCR efficiencies have been determined and two different software programs (NormFinder, qBase) have been used to evaluate expression stability in NE samples from CF patients (n=9) and controls (n=9). DNAs from all individuals were analyzed for CFTR gene. RNA quality was determined and only samples with a RIN above 5.2 were included. The experiment has been carried out twice from two independent RT reactions and each sample was analyzed in triplicate. We have identified three endogenous genes suitable for accurate normalization of CFTR data expression.

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**P01.038****A diagnostic approach for characterization of the CFTR gene defects by mRNA analysis**

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A significant percentage of CF alleles remain unidentified in most population, even after extensive studies of the *CFTR* gene by PCR based procedure. We believe that mRNA analysis may allow researchers to define the pathogenic role of sequence variations not yet defined and particularly splicing defects. After an extensive analysis at DNA level, in a cohort of 745 CF patients, 81 alleles (6%) were still unknown. Aim of this work was to evaluate the role of the CFTR analysis at mRNA level as a diagnostic method for the characterization of molecular defects in CF patients who still had one or two unidentified alleles. RNA was extracted from nasal epithelial cells and collected using cyto-brush from 7 CF patients and 3 non-CF controls. The cDNA was amplified in six overlapping fragments spanning the entire *CFTR* gene. Disease-related mutations were identified in two patients; mRNA analysis performed on two other related patients with a deletion of exon 2 at DNA level, showed two novel transcription products carrying a deletion of exon 2-3 and an insertion of intron sequence of about 80bp near exon 6b, respectively. Two patients presented low level of mRNA product and should be analyzed by quantitative technique. One patient showed a normal profile. In conclusion our data suggest that the defects at RNA level could explain the pathogenic role of abnormal mRNA products in Cystic Fibrosis onset. In our experience, CFTR mRNA analysis represents an effective diagnostic tool for the identification of unknown molecular defects of the *CFTR* gene.

**P01.039****MALDI-TOF based multiplex assay for Cystic Fibrosis newborn screening**

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Cystic fibrosis (CF) is a heterogeneous disease and one of the most common autosomal recessive diseases known in the European population. Since the identification of the responsible gene, *CFTR*, more than one thousand mutations have been identified, most of which are very rare. The number and the selection of mutations tested for vary among laboratories and countries, being common to tailor the mutation panels to local patient population served. Limited sets of mutations, as the ACMG/ACOG 25 mutations and commercially available panels, are insufficiently sensitive for certain groups within a diverse population. We developed and evaluated a MALDI-TOF based mul-

tiplex genotyping assay to detect 185 CF mutations. The methodology is extremely sensitive, allowing the genotyping of very small dried blood spot samples usually employed in newborn screening programs. Furthermore, the Sequenom MALDI-TOF platform is a rapid and high throughput system, allowing to process a high number of samples simultaneously in a cost-effective manner. We validated the system by performing the assay on 348 dried blood samples in the Galician CF newborn screening pilot program, and 75 samples in the American CF proficiency testing (CDC) and the European CF quality assessment scheme (CF European Network). In the 2006-2007 period we detected 11 new CF cases, of which 4 cases would be missed using the ACMG/ACOG minimal panel of 25 mutations.

**P01.040****A retrospective analysis of patients tested for cystic fibrosis mutations in a reference Genetics center in Izmir, Turkey**

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Cystic fibrosis (CF) is an autosomal recessive disorder of epithelial ion transport caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. The *CFTR* gene is located at 7q31.2 and functions as a chloride channel and controls the regulation of other transport pathways. More than 900 mutations and variants have been described in the *CFTR* gene. We have retrospectively evaluated the molecular test results of 550 CF patients referred to the Molecular Genetics Laboratory of Medical Genetics Department, Faculty of Medicine, Ege University, Izmir - Turkey in the last three years. Patients had been tested for 36 mutations in the *CFTR* gene using the strip assay method (Innogenetics, Belgium). Out of 550 patients (1100 alleles) tested, 488 (88.73%) did not carry any mutations, while 27 patients (4.91%) were either homozygous or compound heterozygous, 35 (6.36%) carried only one detected mutation. Allelic frequencies for the six most common mutations in the positive groups were 41.57% (F508del), 14.61% (I148T), 11.24% (2183AA-G), 7.87% (N1303K), 4.49% (W1282X) and 4.49% (R347P). The rest of the alleles (15.73%) showed rare mutations which were 3210+1G-A, 3199del6, 621+1GT, G85E, 2789+5GA, G542X, R117H, 3849+10kbCT. No patient showed the mutations 2184delA, I507del, 1717-1GA, R334W, 3659delC, G551D, 1078delT, R1162X, R560T, A455E, 711+5GA, R553X, Q552X, 394delTT, E60X, 2143delT, 3905insT, CFTRdele2,3(2.1kb), 711+1G-T, 3272-26A-T, 1898+1G-A. In conclusion, the referrals for CF mutation analysis increased annually. The low mutation detection rate may be associated with the physicians' attitudes as they started to use molecular testing in the differential diagnosis of the diseases.

**P01.041****Allelic heterogeneity of glycogen storage disease type III: a study of 34 patients**

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**Introduction**

Glycogen storage disease type III (GSDIII) is due to the deficiency of the glycogen debranching enzyme (AGL). Deficiency of AGL activities causes an incomplete glycogenolysis resulting in the accumulation, in liver and/or muscle of an abnormal glycogen. Clinical symptoms include variable tolerance to fasting, hypoglycaemia and hepatomegaly, frequently accompanied by muscular hypotonia and hypertrophic cardiomyopathy. To date, 71 genetic alterations of AGL gene have been described in GSDIII patients.

**Material**

Here we report the molecular characterisation of 34 GSDIII patients. The 33 AGL gene coding exons were screened by single strand conformation polymorphism and sequenced when an abnormal electrophoretic profile was observed. The allelic distribution of the c.3199C>T and c.3343G>A polymorphisms were determined on 64 control healthy patients by PCR-amplification and enzymatic digestion.

### Results and discussion

In this population 65/68 pathologic alleles were identified, including 21 new mutations. Our molecular study on French GSDIII patients of various ethnic ancestries confirms both the allelic heterogeneity of AGL gene mutations and the strong influence of individual genetic background on genotype-phenotype relationships. Nevertheless in some ethnical groups, some specific mutations were prevalent, probably because of founder effects. As previously reported, we were not able to show genotype-phenotype correlations. Even so we raise the hypothesis of the role of the c.3199C>T and c.3343G>A polymorphisms on the severity of the clinical symptoms. We postulate that these mutations do not confer by themselves GSDIII phenotype, but their association with other disease-causing mutations could accentuate muscular manifestations.

### P01.042

#### Clinical and molecular findings of metachromatic leukodystrophy; A case report of an Iranian family

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Metachromatic leukodystrophy (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.

ARSA is the only gene associated with arylsulfatase A deficiency. This 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 patient was 5 years old boy and was referred with delayed development, mental retardation, loss of speech, urinary incontinence, seizure, spastic quadriplegia and hyporeflexia. EEG was abnormal and brain advanced dysmyelogenesis was reported in MRI. His parents had consanguineous marriage and three similar cases were seen in familial pedigree. The identity of the mutation was confirmed by amplifying all eight exons by PCR 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.

### P01.043

#### Two cases of Morquio IVB type in Bulgaria

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The authors report two cases with Morquio IVB type .The first child present with typical clinical presentation -normal intelligence, dysostosis multiplex.The DMB test showed increased level of MPS.Electrophoresis showed chondroitin sulfate.The diagnosis was confirmed by low beta-galactosidase in leukocytes. The DNA analysis showed the most common in Europe mutation W273L/W273L.The second child present with skeletal anomalies, coarse facial features, astigmatism, myopia, joint hyperflexibility, dyslalia, cortical atrophy with enlarged subarachnoidal spaces on the convex , ventricles and basal cisterns, slightly expressed hypotonia. The electrophoresis showed unidentified oligosaccharides.The enzyme assays -low beta galactosidase in leukocytes and fibroblasts.The DNA -analysis showed two nonpathogenic polymorphisms c.29C>T/heterozygote/, c.34T>C/homozygote/ and a heterozygous state for a common mutation R201H in exon 6 of GLB1 gene and a novel mutation P597S in exon 16 of GLB1 gene.A genetic counseling, carier testing was proceeded to the parents and the brother of the child.

### P01.044

#### Monozygotic sisters with MPS I: a year later

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Mucopolysaccharidose type 1 (MPS1, Hurler syndrome) is an hereditary disease caused by a deficiency of the lysosomal enzyme alpha-L-iduronidase (IDUA; EC 3.2.1.76). Non degraded glycosaminoglycans (GAGs) are stored in the lysosomes and excreted with urine. Last year we reported on a family with monozygotic sisters suffered from MPS1 (EJHG 2007,15(S1):77). The probands are homozygous for Q70X/Q70X. Last year they were under observation by a geneticist, a lung diseases specialist, a neurologist, an orthopedist, an otorhinolaryngologist, a pediatrician and a surgeon. Every specialist found a lot of the pathologic features. The disease was taking a severe progressive course. Clinical symptoms of MPS I become progressively worse. Children's general state is serious. It was possible to launch an enzyme treatment (Aldurazyme® (laronidase). Each child was treated with enzyme infusion five times. After this treatment, both the liver and the spleen enlarged were found to abate their size, however some allergic symptoms appeared. The immunological study has revealed antibodies to Aldurazyme. Probands need a complex follow-up.

### P01.045

#### Niemann-Pick disease, Clinical Course and Molecular Findings; A Case Report of an Iranian Patient

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Niemann-Pick disease (NPD) is an autosomal recessive inherited lipid storage disorder that results from deficiency of the enzyme-acid sphingomyelinase. In people with this condition, this enzymatic defect causes harmful amounts of lipids to accumulate in the spleen, liver, lungs, bone marrow, and brain. Thus patients present with progressive lung disease, hepatosplenomegaly, short stature and pancytopenia. NPD is divided into four main types based on the genetic cause and the signs and symptoms (Niemann-Pick disease type A., B, C & D).

Mutations in the SMPD1 gene cause NPD types A (neurodegenerative form) and B (visceral form). This gene provides instructions for producing an enzyme called acid sphingomyelinase. Mutations in either the NPC1 or NPC2 gene cause NPD type C. The NPC1 gene provides instructions for producing a protein that is involved in the movement of cholesterol and lipids within cells.

The SMPD1 gene consists of six exons located on chromosome 11q and the NPC1 gene contains of 20 exons located on 18q11-q12 are found responsible for the most NPD cases.

A 16 year old girl second of two children born of third degree consanguineous marriage was referred to our clinic. The long term of jaundice, delayed motor milestones, abnormal function of liver and Bone marrow aspiration were showing Niemann Pick's Disease. Using direct-sequencing of the entire coding region and splice junctions of the SMPD1 and NPC1 genes is obtained and analyzed. A G/C mutation at exon 5 was found in SMPD1 gene.

### P01.046

#### Growth and nutritional status of some micronutrients and trace elements in patients with phenylketonuria

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certain micronutrient deficiencies occur either due to rigid therapeutic dietary restriction, aversion to certain food stuffs or due to recurrent episodes of vomiting and diarrhea. Semi-synthetic formula containing low phenylalanine (Phe) content provides the majority of protein and energy in the diet while the rest of phenylalanine are met by low protein natural foods. Restricted intake of high biological value protein, let children with phenylketonuria (PKU) vulnerable to have lower than normal plasma concentrations of certain micronutrients. Aim of the study is to assess the effects of phenylalanine restricted diet on the growth of our PKU patients after one year of dietary management. We also aimed to investigate the nutritional status of the following trace elements and micronutrients; zinc, copper, vitamins C, E, A, and B-carotene among

17 PKU patients following dietary intervention coupled with multivitamin supplementation. Data were compared to those of their matched normal controls. PKU patients have marginal vitamin A deficiency (mean plasma level ;  $25.2+/-6.62\text{ug/dl}$  versus  $48.5+/-10.1\text{ug /dl}$  of control; highly significant ( $p>0.0001$ ). Mean plasma levels of B-carotene was also less than that of controls with a highly significant difference ( $50.65+/-15.37\text{ug/dl}$  vs.  $75.80+/-19.60\text{ ug /dl}$ ;  $p<0.001$ ). Mean plasma levels of zinc, copper, vitamins C and E were comparable to those of controls without statistical significance ( $p<0.05$ ). Physical growth parameters were not significantly different between the two groups. Nutritional care plan for dietary intervention managing PKU children should involve periodic assessment of protein and calorie nutritional status in addition to regular careful evaluation of micronutrients.

#### P01.047

#### The role of Copper metabolism in the clinical and biochemical polymorphism in 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. The activity of other participating enzymes depends on the presence of microelements. Microelements determine the conformation of the protein part of enzymes and their activity. The Copper is included in Phe metabolic way. There is an antagonism between the elements: Cu-Zn. The correlation change of microelements brings to the irregularity of the enzymes' activity.

**Methods:** 30 PKU children presented the troubles of sleep(83%), skin rashes (exudative, maculoerythematous papules, sclerodermic)-63%, tics, convulsions(13%), motor stereotypy(47%), 60% children were absolutely blond. They were investigated for the blood level of Copper, Zinc and ceruloplasmin, along with the level of free amino acids in blood and urine.

**Results:** In addition to the amino acids troubles, the medium level of Copper was  $23.38+/-1.05\text{ }\mu\text{mol/l}$ , the Zinc level  $-10.5+/-0.52\text{ }\mu\text{mol/l}$ , the ceruloplasmin level  $-234.88+/-11.93\text{ mg/l}$ . The low level of Zinc leads to the process when Copper takes the place of Zinc in enzymes, and it leads to functional abnormalities of enzymatic systems containing Zinc with their clinical manifestations.

We added to the traditional diet the drug metabolical correction containing the preparations of Zinc (25-50 mg/day). As the result, the skin rashes, troubles of sleep, motor stereotypy, tics disappeared; the hair color became darker.

**Conclusion:** The effectiveness of the PKU treatment increases if combining the low Phe diet with drug metabolical correction, considering all metabolical components.

#### P01.048

#### Cardiomyopathy the presenting feature pathology in propionic acidaemia

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We describe cardiomyopathy as the presenting feature in two teenage years boys with propionic acidaemia. 15 year old and 13 year old brothers were well and normally active until the occurrence of chest pain and dyspnoea in the elder boy. The parents were first cousins and a 7 year old sister had died of an idiopathic cardiomyopathy 20 years earlier. Mitochondrial vitamin & cofactor cocktail therapy and carnitine therapy did not alter his symptoms; the carnitine level was normal . During his workup an elevated propionyl-carnitine level was detected, similar to that observed in severe neonatal propionic acidaemia. Direct assay of propionyl-CoA carboxylase (pcc) revealed 4% residual activity. Mutation analysis demonstrated that both brothers had G188R and N536D mutations resulting from c.562 G>A and c.1606 A>G. These brothers represent unique cases of propionic acidaemia with initially normal cardiac function. This presentation of metabolic cardiomyopathy may be amenable to medical therapy if detected before irreversible cardiomyopathy has occurred.

#### P01.049

#### Novel mutations in ATP7B gene detected in patients with Wilson disease from Bashkortostan

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Wilson disease is an autosomal recessive disorder of hepatic copper metabolism caused by mutation in the gene encoding a copper-transferring P-type ATPase (13q14.3-q21.1) and leading to heavy hepatic and neurological disorders. The purpose of this research was the analysis of correlation between clinical features (neurological, neuropsychological and liver disorders) and types of mutations.

We observed 71 patients and 96 members of their families from Bashkortostan, using clinical examination, biochemical analyses of blood and instrumental methods (hepatic scanning, hepatography).

We had carried mutation analysis in 28 families with WD from Bashkortostan.

Using SSCP analysis followed by sequencing 19 exons (2,3,4,5,6,7 ,8,9,10,11,12,13,14,15,16,17,18,19,20) of ATP7B gene were identified 8 mutations and 3 polymorphisms in 83,9 % of chromosomes. We have detected 2 novel mutations: Ala718Pro and Lys1315\_Arg-1316delinsGlu. The most common mutation in Bashkortostan were His1069Gln - 48,2% and Lys1315\_Arg1316delinsGlu - 10,7%.We found correlation between type of mutation and clinical manifestations. Patients with new deletion had more severe clinics with early manifestation and hard liver disorders.

#### P01.050

#### Functional characterization of 4 CBS mutations found in homocystinuric patients

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Homocystinuria due to CBS deficiency (MIM#236200) is a rare autosomal recessive disorder characterized by extremely elevated levels of homocysteine (Hcy) in plasma. More than 140 different mutations have been described worldwide and near 1/3 of these mutations have been heterologously expressed and tested for CBS activity. In the Iberian Peninsula and South America the p.T191M mutation is particularly prevalent and accounts for approximately 50-70% of the alleles. The remaining mutations are found in a few pedigrees being mostly private. We had found 3 new mutations (p.M173del, p.P200L and p.D281N) and a previously described one (p.P49L) in four homocystinuric patients (three Spanish and one Indian).

With the aim to assess the pathogenicity of these mutations they were expressed heterologously in *E. coli* and their enzyme activities were assayed in vitro, both in the absence and presence of the CBS activators PLP and SAM. The wild-type CBS activity in the presence of PLP was taken as reference (100%). The expression of the mutant proteins was confirmed by Western-blotting in denaturing conditions. Mutations p.M173del and p.D281N showed null activity and a complete lack of response to the activators, confirming their pathogenicity, whereas p.P49L and p.P200L exhibited activities close to 30% and a strong response to PLP. Furthermore, p.P200L showed good response to SAM. These mutations were found in two patients with a mild phenotype. Mutation p.P49L was found, in combination with p.R125Q, in a 53 year-old male who presented with a stroke and in his asymptomatic 51 year-old sister.

#### P01.051

#### Hereditary hyperferritinemia cataract syndrome: characterization of two mutations in the L-ferritin gene

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Hereditary hyperferritinemia cataract syndrome, an autosomal dominant disease, is characterized by early onset bilateral cataract. Affected individuals show high levels of serum ferritin without iron overload. Elevated serum ferritin results from misregulation of L-ferritin transla-

tion. Ferritin is the major intracellular iron store protein and consists H (heavy) and L (light) subunits which are encoded in chromosomes 11 and 19, respectively. The iron responsive element (IRE) in the 5' untranslated region (5'-UTR) of L-ferritin mRNA interacts with trans-acting iron regulatory proteins (IRPs) to regulate ferritin translation. Mutations in the 5'UTR of the L-ferritin gene, result in reduced affinity for IRPs and subsequently in constitutive L-ferritin synthesis which results in congenital cataract. In the present study, we analyzed two families with early onset bilateral cataract and marked hyperferritinemia. Genomic DNA was isolated from peripheral blood and the IRE L-ferritin gene was completely sequenced. Morphology of cataracts of both families showed different phenotypes. Onset of cataract symptoms ranged from 15 year-old to 40 year-old. No other ocular or systemic anomalies were found to be present. Propositi underwent surgery with satisfactory results. Levels of ferritin serum were compatible with hyperferritinemia. DNA analysis of both families detected two different missense mutations in the loop region of the L-ferritin gene, one of them previously reported, these mutations in the L-ferritin gene result hereditary hyperferritinemia cataract syndrome.

#### P01.052

#### Analysis of the *CTNS* gene in Spanish patients with cystinosis disease

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Cystinosis (MIM #219800) is an autosomal recessive disorder characterized by intra-lysosomal accumulation of cystine due to an impaired transport of free cystine out of lysosomes.

Three phenotypical forms have been described according to the age of onset and severity of the clinical symptoms: infantile, juvenile and ocular nonnephropathic cystinosis.

The gene for cystinosis, *CTNS*, has 12 exons and encodes a 367 amino-acid lysosomal membrane protein called cystinosin. Up to now 84 different mutations have been described.

We have analyzed the *CTNS* exons and intron boundaries, as well as the promoter region in 21 unrelated Spanish cystinosis patients, in order to find possible genotype-phenotype correlations and to enable identification of carriers and prenatal diagnosis. In this study, 11 different mutations were identified, 5 of which are novel. The 57-kb deletion is the most prevalent mutation in our country (33,3% of the alleles), as seen in other studied populations. This deletion together with other 4 mutations (previously described p.T7fsX6, p.T216fsX11, p.G308R and p.M1T which is novel) accounted for 80% of the alleles, which facilitates molecular diagnosis and genetic counselling in this disease.

#### P01.053

#### Characterization of cystinuria double knockout for b<sup>0+</sup>AT and rBAT

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Cystinuria is a common recessive disorder of renal reabsorption of cystine and dibasic amino acids that results in urolithiasis of cystine. Cystinuria is caused by defects in the amino acid transporter rBAT/b<sup>0+</sup>AT. Mutantions in *SLC3A1* (rBAT) cause cystinuria type A, characterized by a silent phenotype in heterozygotes (phenotype I). Mutations in *SLC7A9* (b<sup>0+</sup>AT) cause cystinuria type B, which heterozygotes in most cases hyperexcrete cystine and dibasic amino acids (phenotype non-I). The *Slc7a9* null knockout mouse model (*Stones*) and the *Slc3a1* knockin (*Pebbles*) develop cystinuria B and A respectively. All homozygous mutants hyperexcrete cystine and the three dibasic amino acids, and ~40% of them present cystine calculi in the urinary system. To facilitate in vivo investigation of cystinuria we have generated double knockout mice by crossing the two cystinuria mice models (*Stone* and *Pebbles*) and we have characterized the nine genotype combinations of the F2 in mixed background C57BL/6J-C3H.

Mice were X-ray analyzed at 2.5 and 8 months to appraise calculi formation. Histopathology studies and metabolic cage experiments to collect urine to quantify amino acids excretion were also performed. Preliminary results indicate that double knockout mice has more se-

vere phenotype than single knockouts: single mutants which are heterozygote for the other subunit and double mutants show less viability, higher stone rate and a more severe urinary system damage.

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#### P01.054

#### Single exon deletions in the PAH gene in Polish PKU patients

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The majority of molecular defects causing phenylketonuria (PKU) are missense mutations of the phenylalanine hydroxylase gene (PAH), which are detectable with use of classical molecular techniques basing on single exon amplification, heteroduplex analysis and sequencing. The cumulative mutation detection rate in various groups of PKU-patients reaches 90-99%. However, the above procedure fails to detect single exon deletions.

The aim of this study was to establish simple, non-laborious PCR-based methods for detection of three PKU-causing single exon deletions and to assess their frequency in Polish PKU-patients.

**Methods:** An attempt to detect Ex5del955, Ex5del4232ins268 and EX3del4765 deletions was undertaken with use of published data on the mutated sequence of the PAH gene. DNA samples from 25 PKU Polish patients were analyzed, in whom only one or no PKU-causing mutations (a single case) were identified despite extensive analysis performed with classical methods (in the remaining 245 patients, in whom DNA samples were tested, both mutations were found).

**Results:** PCR protocols for amplification of mutated alleles were successfully established. Following PCR primers were used: Exon5del955for-gcacatttggaaatccacagcaagg,

Exon5del955rev-gtctctagactctaggagtccccag, Exon5del4232for-gttcccatctgtcagttgcctg, Exon5del4232rev-ggaggatctgtccgccttc, Exon3del4765nestedfor-gcattgtccaagtacctgccttg (nested PCR), Exon3del4765for-acaggcacacaccatgc and Exon3del4765rev-gccactatggattgggtgacc. In the tested samples, six cases of Ex5del4232ins268 deletion were found as well as one case of each of the other deletions, which were interestingly detected in the same patient's sample.

**Conclusion:** Ex5del955, Ex5del4232ins268 and EX3del4765 deletions in the PAH gene can be detected with use of simple PCR-based methods and are present in around 2%-3% of Polish PKU-patients.

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#### P01.055

#### Detection of mutations in two families with Ethylmalonic encephalopathy using real-time PCR

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Ethylmalonic encephalopathy (EE, OMIM # 602473) was diagnosed in three children from two unrelated Cypriot families. They presented with neurodevelopmental delay, prominent pyramidal and extrapyramidal signs, recurrent petechiae, orthostatic acrocyanosis and abnormal MRI images. They had persistent lactic acidemia, markedly elevated urinary excretion of ethylmalonic acid, 2-methylsuccinate, isobutyrylglycine and isovalerylglycine, and moderately raised butyrylcarnitine in blood. In one patient muscle cytochrome C oxidase activity was greatly reduced. The diagnosis was confirmed by westernblot analysis in fibroblasts which showed the absence of the ETHE1 protein. Molecular analysis of the *ETHE1* gene was carried out using both PCR and sequencing. The proband of the first family was a compound heterozygote for a deletion in exon 4 and a missense mutation in exon 5, L185R. In order to establish the presence of the deletion in a heterozygous state, we carried out quantitative real-time PCR on DNA extracted from both the patient and his healthy parents. The proband from the second family was homozygous for the deletion in exon 4 and in this case quantitative real-time PCR demonstrated that the parents were both heterozygotes for the deletion. The exon 4 deletion and the

L185R missense mutation, previously reported in EE patients, were shown to be associated with the absence of the ETHE1 protein.

#### P01.056

##### Mutation analysis of Fabry disease in Argentina

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Fabry disease is an X-linked inborn error of glycosphingolipid metabolism, resulting from mutations in the alpha-galactosidase A gene (GLA). Very few reports on mutation analysis for Fabry disease in the Argentinean population are available in literature. Here we report mutation analysis in 16 unrelated Argentinean families with Fabry disease. Methods: Genomic DNA was isolated from affected males and female relatives, and the entire alpha-Gal A coding as well as flanking intronic sequences were amplified by PCR and analyzed by automated sequencing.

Results: Thirteen different mutations were identified, including eight missense mutations (D155H, C174G, C202Y, N215S, Y216C, D264Y, A292T, L415P); two nonsense (R227X, E398X); one splice site mutation IVS4-1G→A and two small deletions: one complex G144fsX15 (del c.431-442, del c.448-459) and K374fsX15 (del c.1122-1125 del AGGA). Six were novel mutations (underlined) and seven were previously described. Mutation analysis provided precise identification of 33 heterozygotes among female relatives and detected a de novo mutation (IVS4-1G→A). Discussion: It is generally assumed that Fabry mutations are private; however this does not seem to be the case of our sample. Mutation L415P was found in four different families presumably unrelated by pedigree analysis. Haplotype analyses with microsatellite markers tightly linked to the GLA gene are being performed in order to define whether this mutation is recurrent in Argentina or the result of a founder effect. These studies further define the heterogeneity of mutations causing Fabry disease and permit precise carrier identification, which has consequences for genetic counselling and for treatment.

#### P01.057

##### Screening for Fabry disease using alpha-galactosidase assay on dried blood discs : a prospective nationwide multicentric study

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Background. Fabry disease (FD, OMIM 301500) is a multisystemic and clinically heterogeneous disease resulting from a deficiency of the lysosomal enzyme alpha-galactosidase A. Hypertrophic cardiomyopathy is a frequent symptom of the classic form of the disease and a cardiac variant, characterized by residual alpha-galactosidase A activity and a milder phenotype, has also been reported. In a prospective nationwide multicentric study, we have studied the prevalence of Fabry disease in a hypertrophic cardiomyopathy referral population.

Methods. We have developed and validated an assay of alpha-galactosidase A activity on dried blood paper discs. Using this method, we have assayed alpha-galactosidase A activity in 330 adult patients affected with hypertrophic cardiomyopathy who had never been diagnosed with FD.

Results. Three patients (0.9%) were found to exhibit low alpha-galactosidase A activity in dried blood spots. This was confirmed by a second determination of alpha-galactosidase activity in leukocytes. The three patients presented neither the typical clinical signs of classic FD, such as acroparesthesias or hypohidrosis, nor kidney insufficiency, suggesting a variant form of FD. All patients with low alpha-galactosidase activity had

mutations in the GLA gene. DNA sequencing also allowed the identification of two additional hemizygotes (half-brothers of the proband) in one family.

Discussion. Screening for Fabry disease should be considered in all cases of unexplained hypertrophic cardiomyopathy. Its recognition is important since specific enzyme replacement therapy is now available. The proposed novel filter paper method favours the collection, storage and shipment of samples. It is simple and efficient for screening programs.

#### P01.058

##### An update in the molecular analysis of classical galactosaemia in Spain and Portugal: Eight new mutations in seventeen new patients

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Classical galactosaemia is an autosomal recessive inherited metabolic disorder due to mutations in the galactose-1-phosphate uridylyltransferase gene (GALT).

We previously reported molecular analysis of 83 Spanish and Portuguese galactosaemic patients. Here we present the molecular results of another seventeen unreported affected individuals.

Thirteen patients of Spanish origin were analysed. We detected six alleles carrying p.Q188R, accounting for 23%. Other six alleles (23%) were identified with the mutation p.K285N. Remarkably, the two patients that were homozygous for this change were of North African origin. We also identified seven novel mutations: p.Q9X, c.328+2T>C, c.328+33G>A, p.I170N, p.C180F, p.V233L, p.P257L. Taking into account all the Spanish galactosaemic diagnosed patients, mutation p.Q188R is still the most frequent mutation identified (43.7%). The second most frequent mutation is p.L195P (13.3%) followed by p.K285N (12.5%).

Four new Portuguese patients were analysed. In four alleles p.Q188R was detected, representing 50%. One novel mutation was identified, p.F171C. Mutations p.L195P and p.K285N still remain undetected in Portuguese patients. In the whole group of 36 Portuguese patients we have analysed until now, mutation p.Q188R remains the most frequent identified (57%). It is worth mentioning that this is the only frequent mutation as the rest of changes identified were found only in one or two alleles each.

Our results confirm the already published observation that p.Q188R is the most frequent mutation in Iberian Peninsula galactosaemic patients (48.5%).

Moreover, our molecular analyses on these seventeen new galactosaemic patients provide eight novel mutations to the database with more than 200 disease-causing mutations already reported.

#### P01.059

##### Normal HPRT coding region in complete and partial HPRT deficiency.

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Lesch-Nyhan syndrome is an X-linked recessive inborn error of metabolism due to a virtually complete lack of hypoxanthine-guanine phosphoribosyltransferase (HPRT) activity (OMIM 300322). Partial deficiency of HPRT (OMIM 300323) is characterized by the effects of excess uric acid synthesis and a continuum spectrum of neurological manifestations, without the manifestations of full-blown Lesch-Nyhan syndrome. Both diseases have been associated with mutations in the HPRT gene. These mutations are heterogeneous and disperse throughout the entire HPRT gene. In 2005 Dawson et al described, for the first time, an individual with gout in whom HPRT deficiency appeared to be due to a defect in gene regulation.

Patients and methods: Four patients with partial HPRT deficiency and one patient with Lesch-Nyhan syndrome were studied. The RNA and RNA-free genomic DNA samples were isolated from whole blood. Analysis of HPRT coding region was performed by Reverse transcription of patients RNA, amplification of HPRT cDNA by PCR and automated sequencing. HPRT mRNA expression was determined by Real-Time PCR technology. All nine exons of the human HPRT gene, its intronic flanking sequences, and gene regulatory sequences were amplified and automated sequenced. Results: All patients showed a nor-

mal HPRT coding sequence. Determined by mean of Real-Time PCR technology, these patients showed markedly decreased HPRT mRNA expression. Finally, we have analysed genomic regulatory sequences from HPRT gene in these patients but no mutation was found.

Conclusions: This is the first report of a patient with Lesch-Nyhan syndrome due to a defect in HPRT gene expression regulation.

#### P01.060

#### Clinical, biochemical, and molecular diagnosis of L-2-hydroxyglutaric aciduria; Report of three Iranian families with six affected cases

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Background: L-2-Hydroxyglutaric aciduria is a novel autosomal recessive neurometabolic disorder. First described by "Duran" in 1980 about 100 cases have been reported. It is characterized by slowly progressive neurological dysfunction with cerebellar ataxia, pyramidal sign, intellectual decline, seizure etc.. MRI scanning is highly characteristic and screening for organic acid(L-2-Hydroxyglutaric acid) in the urine, serum, and CSF is diagnostic.

Materials & Methods: We have investigated three Iranian families with six affected children aged (4 to 16 years) who were suspected for this rare disorder, by urine organic acid assay and MRI scanning. In two families we analyzed the Duranin gene which is responsible for hydroxy-glutarate dehydrogenase.

Results: Affected cases were evaluated because of clinical findings. Urine levels of L-2-Hydroxyglutaric acid were strongly increased. MRI scanning of the brain showed hyper intense signal on T2 weighted images of the sub-cortical white matter, and basal ganglia in all of the patients. We have identified the mutation in one of the families. It was a large deletion encompassing at least exons 7 and 8, the process is ongoing yet. In this family, because the mother became pregnant we did PND, unfortunately the fetus was affected in homozygote state. Conclusions: Because of its inheritance pattern(autosomal recessive), and high rate of consanguineous marriages in Iran, the prevalence of this disorder might be higher, between mentally handicapped patients, especially those with macrocephaly. So we should consider this rare entity in our differential diagnosis.

#### P01.061

#### Lysosomal membrane permeabilization triggers cathepsin D secretion and cell death in lysosomal storage diseases

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Lysosomal storage diseases (LSDs) are a group of inherited metabolic disorders due to the defective activity of lysosomal hydrolases and characterized by accumulation of undegraded substrates in the lysosomes, leading to peripheral organ cell damage, neurodegeneration, and premature death in most cases (Saftig et al, 2005). Here we have used our mouse model for Multiple Sulfatase Deficiency (MSD) (Settembre et al, 2007), to study the intracellular signals triggered by intralysosomal storage. Using fluorescent weak bases to examine the acidic lysosomal compartment from isolated splenocytes and mouse embryonic fibroblasts, we have found an increased lysosomal membrane permeabilization (LMP) in MSD cells compared with their wild-type counterparts. These results suggest a loss of the intralysosomal acidic pH that can be mimicked in wild-type cells by the addition of the specific inhibitor of vesicular proton pump, Baflomycin A1. Lysosomal destabilization in MSD cells correlates with several hallmarks of apoptosis such as lysosomal secretion of the aspartate protease cathepsin D, loss of mitochondrial membrane potential, release of cytochrome C, activation of caspase-3, and annexin V staining. Interestingly, LMP seems to be a common sign of other LSDs as suggested by the increased LMP observed in splenocytes from a mouse model of Hunter disease. Together, these evidences support the role of LMP and the release of lysosomal proteases in the initiation and execution of apoptosis in pathological situations such as LSDs.

#### P01.062

#### Mutational spectrum and phenotype-genotype correlation in Spanish Methylmalonic Acidemia with Homocystinuria, cbIC type

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Methylmalonic acidemia with homocystinuria, cbIC type is the most frequent genetic disorder of vitamin B<sub>12</sub> metabolism. We present the mutational spectrum of 22 patients, classified belonging to the cbIC complementation group by biochemical and cellular approaches, to provide insight on the phenotype-genotype correlations and on the search for new therapeutic targets. The mutational spectrum included five previously described mutations (M1L, R91fs, R132X, R153X, R161X) and one new splicing change (IVS1nt2T>G). The most frequent change was the known mutation R91fs caused by a single nucleotide duplication in position 271 which accounted for 82% of the mutant alleles characterized and 77% of the cbIC in homozygous fashion. Up to date only some family studies have been performed to rule out the presence of a big deletion. The frequency of c.271dupA is higher than described in other studied populations such as the Italian and the Portuguese cases. These results allow the confirmation of the disease in our population by specific mutational analysis. The other changes were rare mutations identified in one or two alleles (2 to 4%). All the mutations found produce a premature truncation codon. The majority of the patients exhibited a neonatal severe presentation. Although most patients responded biochemically to B12 they exhibited a fatal outcome. Further studies of the physiopathology of the disease to provide insight about the severity of the symptoms despite of B12 responsiveness as well as the in vitro use of drugs to read-through the PTC mutations will be discussed.

#### P01.063

#### Identification of 4 novel mutations in the *NPC1* gene in Spanish patients with Niemann-Pick type C disease

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Niemann-Pick type C (NPC) is a rare autosomal-recessive lysosomal storage disorder characterised by severe progressive neurological deterioration. The disease is caused by mutations in the *NPC1* or *NPC2* genes. Approximately 95% of patients bear mutations in the *NPC1* gene, which encodes a late endosomal integral membrane glycoprotein, and about 5% of the patients show mutations in the *NPC2* gene, encoding a small soluble lysosomal protein with cholesterol-binding properties. Both genes are involved in cholesterol and glycolipid trafficking and transport, so NPC patients show accumulation of unesterified cholesterol and glycosphingolipids in the lysosomal/late endosomal system.

A mutational analysis was carried out in 5 non-related individuals affected by this disorder. Genomic and cDNA from the patients were amplified and sequenced. All the *NPC1* mutant alleles were identified; 4 of them have not been described before and were not found in 100 control alleles. The new changes were: one missense mutation: p.F1079S (c.3236T>C); one nonsense mutation: p.E1089X (c.3265G>T) and two intronic changes that affect the splicing process: c.2604+5G>A in the donor splice site of intron 17 which promotes skipping of exon 17 and c.1554-1009G>A that is located in intron 9 and results in the incorporation of 194 pb of intron 9 in the cDNA as a new exon. Some of the mutant alleles caused degradation of the mRNA by the nonsense-mediated RNA decay mechanism. To identify these mutations, cycloheximide treatment of cultured fibroblast was required.

The financial support from Fundación Niemann-Pick España and from CIBERER (INTRA/07/720.1) is acknowledged.

**P01.064****Isolation and characterization of a novel CHO cell mutant defective in peroxisome biogenesis factor, PEX16, containing aberrant huge peroxisomes**K. Ghaedi<sup>1,2,3</sup>, Y. Fujiki<sup>4,5</sup>,<sup>1</sup>Biology Dept., Isfahan, Islamic Republic of Iran, <sup>2</sup>Royal Institute, Isfahan Research campus, Isfahan, Islamic Republic of Iran, <sup>3</sup>Biology Dept., Faculty of sciences, Kyushu University, Fukuoka, Japan, <sup>4</sup>Biology Dept., Faculty of Sciences, Kyushu University, Fukuoka, Japan, <sup>5</sup>JST, SORST, Tokyo, Japan.

Peroxisomal Biogenesis Disorders (PBDs) are characterized as the congenital cerebro-hepato-renal disorders which are mostly due to the defects in the biogenesis of peroxisomes. In order to study molecular mechanisms of peroxisome biogenesis, CHO-K1 cells have been widely used by our group as the best model cells. Using CHO-K1 cells stably transformed dually with *PEX2* (to prevent frequent isolation of pex2 mutant cells) and EGFP fused downstream of N-terminally 40 amino acid residues of Pex3p, termed TkaEG3-40 cells, we have started a procedure of mutagenizing with MNNG as a chemical mutagenic component and following culture in the presence of P9OH with subsequently an exposure to UV. By the above approach, we have isolated several CHO cell mutant cell lines defective in peroxisome biogenesis which were resemble to the patients' fibroblasts of the PBDs. One of CHO cell mutant colonies which, showed to have morphologic aberrant huge peroxisomes but very few in numbers, termed ZPEG301 cells. ZPEG301 cells belonged to the *PEX16* complementation group as numerous punctuate structures of peroxisomes were restored after its cDNA transfection in to those cells. Moreover temperature shift assay to permissive temperature at 30 degrees of centigrade showed a moderate increase in peroxisomal structures in ZPEG301 cells which is presumably due to the stabilization of peroxisomal membrane proteins such as PMP70. Biochemical pattern of peroxisomal proteins have documented our hypothesis. Taken together these results demonstrate that Pex16p is involved in morphological control and division of peroxisome in mammals besides of its main role in peroxisomal biogenesis.

**P01.065****Molecular diagnostics of phenylketonuria**E. Polák<sup>1</sup>, A. Ficek<sup>1</sup>, L. Kádaš<sup>2</sup>,<sup>1</sup>Comenius University, Faculty of Natural Sciences, Department of Molecular Biology, Bratislava, Slovakia, <sup>2</sup>Institute of Molecular Physiology and Genetics, Slovak Academy of Science, Bratislava, Slovakia.

Phenylketonuria (PKU) is an autosomal recessive inherited disorder arising from the deficiency of phenylalanine hydroxylase (PAH), which catalyses the essential conversion of phenylalanine (Phe) to tyrosine (Tyr). In the majority of cases, PKU is caused by mutations in the *PAH* gene, and it presents with different phenotypes which are classified according to Phe tolerance. More than 500 mutations have been described world-wide and the PAH enzyme has been fully characterized. The incidence of the disease in the Slovak population had been estimated to be 1:10 000 newborn. Seven causative mutations which have been so far identified make up 70 % of all PKU alleles. Thus the aim of our work was to carry out a complete mutation analysis in a sample of 48 unrelated PKU patients with 1 or with no known mutation. The DH-PLC (Denaturating High Performance Liquid Chromatography) method was applied for screening all 13 coding exons of the *PAH* gene. Identified heterozygous fragments were subsequently sequenced to characterize the DNA variants. Sequencing revealed 16 mutations (of which 1 is new) not previously found in the Slovak population, and 2 frequent polymorphisms in the coding region of the *PAH* gene. Corresponding frequencies for all mutations were estimated, and the classification according to phenotypic categories of PKU was identified.

**P01.066****Assessment of the severity of the PAH gene mutations detected in Serbian population: genotype-phenotype correlation and functional studies in vitro**M. Stojiljkovic<sup>1</sup>, B. Perez<sup>2</sup>, L. R. Desvia<sup>2</sup>, C. Aguado<sup>2</sup>, M. Ugarte<sup>2</sup>, S. Pavlovic<sup>1</sup>,<sup>1</sup>Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia,<sup>2</sup>Centro de Biología Molecular Severo Ochoa, Madrid, Spain.

Phenylketonuria (PKU) is caused by deficiency of hepatic enzyme, phenylalanine hydroxylase (PAH). Although numerous factors contribute to the PKU phenotype, several studies showed that mutations in

the PAH gene are main determinant of PKU severity. In the study on 34 unrelated patients with phenylketonuria from Serbia, we identified 19 disease-causing mutations: L48S, R408W, P281L, E390G, R261Q, R158Q, I306V, IVS12+1G>A, Q20X, R111X, V177L, P225T, R261X, L15/S16fsCTdel, S231F, R252Q, R297H, IVS10-11G>A and R413P. According to pretreatment serum phenylalanine level, patients were assigned to classic PKU (65%), mild PKU (35%) and MHP (0%). The most frequent mutation in Serbia is L48S. Since genotype-phenotype correlation inconsistency for L48S mutation has been previously reported, we investigated genotypes involving L48S and their correlation with phenotypes. The homozygous patient for L48S had classic PKU. Also, in combination with null mutations, L48S was associated with severe phenotype. Our findings on the effect of other mutations were mainly in concordance with previous European studies. However, functional and structural effect of the S231F mutation was not previously analyzed. Therefore, we characterized S231F PAH protein in prokaryotic and eukaryotic expression systems. In both systems the mutant enzyme was unstable. Its residual enzyme activity was lower than 10% showing that S231F is a severe mutation. We have found no GroESL chaperone effect and slightly positive effect of the BH4 on the stabilization of the protein structure. These findings elucidated severe phenotype of the patient with L48S/S231F genotype. In conclusion, we showed that L48S is consistently severe PAH gene mutation in Serbian population.

**P01.067****Distribution of Xmn I alleles at the phenylalanine hydroxylase gene in Republic of Moldova**A. P. Gavriliuc<sup>1</sup>, S. A. Groppa<sup>2</sup>,<sup>1</sup>National Center of Reproductive Health & Medical Genetics, Chisinau, Republic of Moldova, <sup>2</sup>State University of Medicine & Pharmacology, Chisinau, Republic of Moldova.

Phenylketonuria (PKU) is a common autosomal recessive disease of amino acid metabolism caused by phenylalanine hydroxylase (PAH) deficiency. The human PAH gene has been localized to 12q22-24, comprises 13 exons spread over 90 kb of genomic DNA (Lidsky, 1984). The complete 2,4 kb cDNA (Kwok, 1985) can be used to detect ten different RFLPs located within the PAH locus.

Genomic DNA was extracted and examined by standard procedures from 68 families with classical PKU, i.e. 272 parental chromosomes. PCR amplification of a 205 bp fragment containing the Xmn I RFLP site was performed as described previously by Goltssov (1992). A total 136 mutant and 140 normal chromosomes were analyzed for Xmn I polymorphic restriction site (GAANN/NNTTC) at the PAH gene region. Frequencies of normal alleles in population Republic of Moldova (0,593; 0,407) are not significantly different from European populations (0,618; 0,382) ( $\chi^2 = 0,2$ ;  $p > 0,7$ ). The level of observed heterozygosity in population of Moldova (0,48) is similar with European countries (0,47). The distribution of mutant PKU alleles (0,772; 0,228) in our study is differs significantly from those observed in European (0,654; 0,346) ( $\chi^2 = 4,9$ ;  $p < 0,05$ ). The Xmn I alleles in our populations had a significant difference in the distribution among normal and mutant chromosomes ( $\chi^2 = 9,25$ ;  $p < 0,01$ ). Frequency of informative cases by RFLP-analysis of Xmn I alleles from PKU families is 32,6% that provide a tool for molecular diagnosis of these disease and carrier status in Republic of Moldova.

**P01.068****Mutational analysis of 11 Spanish Gangliosidosis GM2 (Sandhoff disease) patients**N. de Olano<sup>1</sup>, A. Chabas<sup>1</sup>, M. J. Coll<sup>2</sup>,<sup>1</sup>Institut de Bioquímica Clínica, Hospital Clínic. CIBERER, Barcelona, Spain.

Sandhoff disease (OMIM #268800) is a recessively inherited neurodegenerative disorder in which the catabolism of ganglioside GM2 is impaired, leading to its intralysosomal accumulation primarily in neurons. The underlying genetic defect resides in the *HEXB* gene encoding the  $\beta$ -subunit of  $\beta$ -hexosaminidase. We analyzed the *HEXB* exons and intron boundaries in 11 unrelated affected Spanish patients.

We found 11 different mutations, five of which were new. The novel changes detected, all corresponding to acute phenotypes, include a one-base deletion (c.171delG), two nonsense changes (p.R170X and p.W514X), a missense substitution (p.R533C), and an in-frame deletion of 6 amino acids (c.800\_817del). In six patients, we identified six

mutations, all of them previously described in the literature: two juvenile patients, one of which was homozygous for p.R505Q and the other compound heterozygous for c.1509-26G>A; ,and four infantile patients homozygous for p.P417L, p.Y266D, c.782\_785delCTTT and c.1305\_1306delAG, respectively. Overall our findings point out to 3 particularly interesting aspects of the mutational spectrum of the Spanish population: Null frequency of the common 16 Kb deletion at 5' of the gene, an outstanding degree of homozygosity ( 72% of patients) and a remarkable prevalence of an allele carrying a deletion of a single nucleotide (c.171delG) which accounts for 27% of total mutant alleles.

#### P01.069

##### Mutation analysis in Spanish and Moroccan Patients with Mucopolysaccharidosis IIIC (Sanfilippo C Syndrome)

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Mucopolysaccharidosis (MPS) describes any inherited lysosomal storage disorder resulting from inability to catabolize glycosaminoglycans. MPS III, or Sanfilippo Syndrome, is the type of MPS that results from a deficient heparan sulfate degradation and presents an autosomal recessive inheritance. Clinical symptoms are similar for all types of MPS III and they are due to the lysosomal storage of heparan sulfate. The most important feature is the progressive and severe deterioration of the central nervous system during childhood.

HGSNAT is the gene responsible for MPS IIIC that has been recently identified. It encodes the acetyl CoA:α-glucosaminide N-acetyltransferase, which is a lysosomal membrane protein required to N-acetylate the terminal glucosamine residues of heparan sulfate. To date, about forty mutations have been described in MPS IIIC patients.

In this study we have identified the mutant alleles in five Spanish and one Moroccan patients. A total of six different alleles have been found in this mutational analysis, five of which are novel. One of these alleles contains a double mutation (c.318+1G>A, p.P265Q) previously described, three alleles bear splice-site mutations (c.456-2A>G, c.717+1G>A and c.1462-1G>A) and the other two contain missense mutations (p.G452V and p.L473P). Furthermore, two new possible single nucleotide polymorphisms (SNP) have been identified in intron 1 and intron 5, respectively. Samples from three new patients, one Spanish and two Moroccan, have recently arrived and are currently being analyzed.

This work is being carried out in collaboration with the MPS España. The financial support from this association is acknowledged.

#### P01.070

##### Novel mutation in Tay-Sachs (HEXA gene)

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Tay-Sachs disease is a fatal genetic autosomal recessive disorder, most commonly occurring in children, those results in progressive destruction of the nervous system. Tay-Sachs is caused by the absence of a vital enzyme called hexosaminidase-A (Hex-A). Tay-Sachs disease, the prototype hexosaminidase A deficiency, is characterized by progressive weakness, loss of motor skills, decreased attentiveness, and increased startle response beginning between three and six months of age with progressive evidence of neurodegeneration, including seizures, blindness, spasticity, eventual total incapacitation, and death, usually before age four years. The infant gradually regresses, and is eventually unable to crawl, turn over, sit or reach out.

The HEXA gene provides instructions for making one part of enzyme called (beta-hexosaminidase A). Specifically, the HEXA gene carries instructions for the alpha subunit of this enzyme. One alpha subunit joins with one beta subunit (produced from the HEXB gene) to form a functioning enzyme. Mutations in the gene coding for the β subunit lead to a deficiency of both the HEX A and HEX B form of the enzyme .More than 100 mutations that cause Tay-Sachs disease have been identified in the HEXA gene. The HexA gene is located on the human 15q23-q24 chromosome.

In present study DNA was received from a consanguineous couple who had 1 affected child from who no sample was available. For each

sample we performed genetic analysis of HEXA gene by direct sequence analysis. It was found heterozygote delG mutation in exon 14 of HEXA (β subunit).

#### P01.071

##### Mutations in the urocanase gene *UROC1* cause urocanic aciduria

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The urocanase deficiency (MIM 276880) is a rare disease with only two reported cases, due to the abnormal activity of the urocanase enzyme (histidine pathway) that catalyzes the transformation from urocanic acid to formiminoglutamate (FIGLU). The biochemical hallmark is the urocanic aciduria. We have investigated the urocanase gene *UROC1* gene in a girl presenting ataxia with mental retardation (IQ = 54) and extremely high levels of urocanic acid in urine, ranging between 158-202 mmol/mol creatinine (normal values below 10 mmol/mol creatinine). The genetic analysis of the *UROC1* gene revealed that the propositus is a compound heterozygote for the mutations p.L70P (c.209T>C) and p.R450C (c.1348C>T). Her healthy father was heterozygous carrier of the p.R450C mutation. *In silico* analysis showed that the L70 residue could form part of an α-helix, and then the change to proline may disrupt the α-helix, possibly resulting in an alteration of the structure of the N-terminal region. The R450 residue forms a salt-bridge with the urocanate, substrate of the urocanase. Presence of an arginine residue as the consequence of the mutation would interrupt the urocanase interaction with urocanate. Consequently, the *in silico* predictions suggest that both mutations in the *UROC1* gene, p.L70P and p.R450C, are pathologic. We suggest that urocanase deficiency or urocanic aciduria is a Mendelian disorder of the histidine metabolism caused by mutations in the *UROC1* gene. The toxic effect of the urocanic acid and also, the possible folate deficiency would play a notable role in the physiopathology of this rare condition.

#### P01.072

##### Mutation screening in genes coding for ATP7A, ATP7B and ATOX1 in Czech patients with Menkes disease and Wilson disease

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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 130 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). 14 mutations were found in the ATP7B gene (including prevalent H1069Q mutation, and the newly found A1135T 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- 38.8% of analysed al-

leles- is in accordance with its occurrence in Central Europe. Supported by Grants IGA MZ NR9406, NR9215, MSMT 1M0520 and GAUK 109/06.

#### P01.073

#### The molecular-genetic analysis of ATP7B gene at the Russian patients with Wilson disease

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Wilson's disease (hepatolenticular degeneration) is severe hereditary autosomal recessive disorder, representing combined injury of internal, first of all a liver, and a brain (the basal ganglia), that is caused by accumulation in their cells of copper. Defect of copper-transporting ATPase P-type, being a product of gene ATP7B responsible for development of Wilson disease, leads to abnormalities of inclusion copper into ceruloplasmin, responsible for its export.

The purpose of our investigation is search and determination of frequencies of mutations in ATP7B gene on materials of the Russian patients with the diagnosis «Wilson disease». In research the method of the SSCP-analysis with the following direct sequencing was used. Search and identification of mutations was spent at 104 unrelated probands (208 chromosomes). The frequency of the most frequent for the Russian population mutations in exon 14 (H1069Q) and exon 15 (c.3400delC) amounted at 40,4 % and 2,4 %, respectively.

Eight rare mutations were found in exon 5 c.1744\_1745delAT at one patient, and c.1770insT at three patients; in exon 7 - IVS7DS+3a>g at one patient; in exon 8 - c.2304\_2305insC at one patient; in exon 14 - E1064K at one patient and c.3083\_3085delAGA>G also at one patient; in exon 15 - Gly1111Asp at one patient; and in exon 18 - c.3888delC also at one patient. All the rare mutations listed above have been detected in a heterozygous state. Mutations c.1770insT, c.2304\_2305insC and c.3888delC have been identified for the first time. Also six polymorphisms were identified: c.1-75C>A, c.1-122ins-GCCGC, c.1366G>C, K832R, Arg952Lis, A1140V.

#### P01.074

#### Preliminary results of Neonatal Screening program for Wilson disease in Greece and reporting of novel variants in ATP7B gene

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Wilson disease (WND) is an autosomal recessive genetic disorder and heterozygous carriers do not show symptoms of the disease. WND is caused by mutations in the ATP7B gene and exhibits substantial allelic heterogeneity. WND is observed with a prevalence of approximately 1:30000, a carrier frequency of 1 in 90 and is similar among many ethnic groups.

However the gene frequency is increased in the Sardinian population and the Island of Grand Canaries. In these populations specific mutations reach high frequencies due to inbreeding and founder effects. Preliminary neonatal screening results have also shown increased carrier frequencies in certain parts of Greece. In order to estimate the exact carrier frequencies in areas with increased incidence of disease a neonatal screening program was established. Out of a 100 newborns tested, for the two commonest mutations (H1069Q and R969Q), from the Island of Kalymnos we identified 89 normal, 11 carriers and 2 asymptomatic patients. Taking into account the carrier frequency for the general population there is a statistically significant increase in this area with a p value of 0.07 (Pearson's Chi Square). The method used for the neonatal screening is a capture down protocol designed on Nanochip® 400 System.

We also report the identification of 6 novel variants in the ATP7B gene [p.I930M and p.V1036I (ex.12), p.Y1464S (ex.21), p.N1128Y (ex.15), c.4125-23A>G (IVS21), and c.1544-12C>T (IVS3)], identified by DGGE analysis and direct sequencing. The effects of these mutations were assessed in combination with the clinical phenotype and using *in silico* analysis ("PolyPhen" and "SIFT").

#### P01.075

#### ABCD2 peroxisomal transporter in the metabolism of long-chain saturated and w9 monounsaturated fatty acids

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ABCD2 peroxisomal transporter in the metabolism of long-chain saturated and w9 monounsaturated fatty acids

Peroxisomes are essential organelles exerting key functions in fatty acid metabolism, such as the degradation of very long-chain fatty acids (VLCFAs). VLCFAs accumulate in X-adrenoleukodystrophy (X-ALD), a disease caused by a deficiency of the ABCD1 peroxisomal transporter. Its closest homologue, ABCD2, exhibits a high degree of functional redundancy on the catabolism of VLCFAs, being able to prevent X-ALD-related neurodegeneration (Pujol A. et al. 2004, Hum Mol Genet 13:2997-3006). In search for specific roles of ABCD2, we screened fatty acid profiles of mutant mice lacking Abcd2 in adrenal glands, spinal cord and sciatic nerve, the target tissues of these mice (Ferrer et al.(2005) Hum Mol Genet 14(23):3565-3577), and in liver after different conditions such as fasting and feeding with a VLCFA-rich diet, by gas-liquid chromatography . Our results indicate that ABCD2 plays a role in the degradation of long-chain saturated and w9-monounsaturated fatty acids, enlarging the spectrum of functions attributed to date to peroxisomes.

#### P01.076

#### Mitochondria depletion underlying neurodegeneration in X-ALD?

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X-linked adrenoleukodystrophy (X-ALD) is the most frequent inherited monogenic demyelinating disease (minimal incidence 1:17,000). X-ALD leads to death in boys or to motor disability in adults (adrenomyeloneuropathy or AMN). The disease is caused by loss of function of the ABCD1 gene, a peroxisomal ATP-binding cassette transporter, which function is related to defective β-oxidation of very long-chain fatty acids (VLCFA) in peroxisomes. Therefore, a hallmark of the disease is the accumulation of VLCFA in plasma and tissues. The mouse model for X-ALD (Ald knock-out) exhibit a late-onset phenotype closely related to adrenomyeloneuropathy, with neurodegenerative features beginning at 15 months of age (1,2). Using microarrays, Q-PCR and Western Blots of mouse spinal cords, we have identified a mitochondria depletion as very early event in the pathogenesis (3.5 months of age). RIP140 (Receptor interacting protein 140), a nuclear co-repressor of mitochondria biogenesis, is upregulated in spinal cord from Aldko mice. VLCFA are able to induce RIP140 in *ex vivo* organotypic spinal cord slice culture. We have also observed a mitochondria depletion in the affected white matter of CCALD (Cerebral Childhood ALD) and cerebral AMN compared to control individuals. This depletion is correlated to a higher level of RIP140 in the non-affected white matter of X-ALD patients. This could be disease causative, or at least, contribute significantly to the neurodegenerative cascade in X-ALD physiopathogenesis.

(1) Pujol et al, Hum Mol Genet. 2002 Mar 1;11(5):499-505; (2) Pujol et al, Hum Mol Genet. 2004 Dec 1;13(23):2997-3006

#### P01.077

#### Molecular analysis of renal hypouricemia in Japan: evidence of a single origin for a common mutation G774A in SLC22A12

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Renal hypouricemia is an inherited and heterogeneous disorder characterized by impaired tubular uric acid transport. Impairment of URAT1, the main transporter for uric acid reabsorption at the apical membrane of the renal tubules, causes renal hypouricemia. G774A mutation in SLC22A12 encoding URAT1 predominates in Japanese renal hypouricemia. We investigated whether the uncertain predominance of hypouricemia on survival or some effects such as founder effect leads to the expansion of G774A mutation in SLC22A12 in Japanese despite of the complications, nephrolithiasis and exercise-induced acute renal failure. Molecular analysis have been undertaken in sixty-nine Japanese renal hypouricemic patients and haplotypic analysis in 31 patients with homozygous G774A mutation using flanking 13 markers (12 single nucleotide polymorphisms and a dinucleotide insertion/deletion locus) around G774A locus. The sharing of the ancestral haplotype clearly implied a common origin of the mutation, the age of that being estimated to be approximately 6,820 years (95%CI 1,860 - 11,760 years, median 2,460 years). The age indicates that the origin of G774A mutation dates back from the time when Jomon people predominated in Japan to the time when Yayoi people started to migrate to Japan from the Korean peninsula. These data are consistent with a recent finding that G774A mutation was also predominant in Korean hypouricemic subjects and indicate that the Asian continent was the origin of G774A mutation. The G774A mutation in Japanese hypouricemia subjects had been brought by immigrant(s) from the continent and expanded in Japanese population either by founder effect or genetic drift (or both).

#### P01.078

#### Microarray Resequencing Analysis of Fumarylacetoacetate Hydrolase (FAH) Gene in Tyrosinemia Patients

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Hereditary tyrosinemia type I (OMIM: 276700) is an autosomal recessive disease caused by defects in fumarylacetoacetate hydrolase (FAH) gene involved in the last step in tyrosine catabolic pathway. To date, over forty pathogenic mutations were described in the FAH gene.

In this study, a total of 22 Turkish tyrosinemia patients were genotyped to detect disease causing mutations in FAH by our design of a resequencing microarray. Our microarray was designed to sequence all exonic and their flanking intronic sequences of the FAH gene to detect sequence variations. In brief, FAH gene has been amplified from genomic DNA by short or long range PCR. After purification, all PCR products were quantitated and equimolar amount of them were pooled. After the fragmentation step, fragmented PCR products were end labeled using a biotin-labeling reagent and hybridised with DNA arrays. Then, arrays were processed by steps of washing and staining on fluidics station. Scanned arrays were analyzed using Affymetrix GeneChip Resequencing Analysis Software.

Mutation screening results showed that 12 different type of mutations were responsible of disease development in our cohort of Turkish tyrosinemia patients. Four different polymorphisms were also described in our patients. Detected mutations by DNA resequencing chip technology were also reevaluated and confirmed by direct DNA sequencing. To our knowledge, this work is the first report showing the application of DNA resequencing chip as rapid and reproducible mutation screening tool for genetic analysis of patients with hereditary tyrosinemia . (Supported by State Planning Organisation DPT2006K1206400603).

#### P01.079

#### Molecular and clinical findings in two Coffin-Lowry syndrome patients

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Coffin-Lowry syndrome (CLS, MIM#303600) is an X-linked semidominant disorder. Cardinal features in males include mental retardation, facial dysmorphism, digital tapering, and progressive spinal deformity. In females the intensity of symptoms is variable. The estimated frequency is 1:50000 - 1:100000 births. CLS is caused in the majority of cases by mutations of the RSK2 gene (*RPS6KA3*), which maps to Xp22.2 and is split into 22 exons. The gene encodes for a serine/threonine kinase, RSK2, acting at the distal end of the MAPK/ERK signaling pathway.

We present two Polish patients with the clinical diagnosis of Coffin-Lowry syndrome confirmed on the molecular level. Patient 1 is a boy aged 4 years. He has moderate mental retardation, hypotonia, facial dysmorphism, digital tapering, dental anomalies, spinal deformities, seizure and cataplexia. Molecular analysis of the RSK2 gene revealed the presence of a substitution G to A in the first position of intron 10 (c.845+1G>A). The mutation appeared *de novo*. Besides, a polymorphism in exon 10 (c.798C>A) was identified. Patient 2 is a girl aged 9 years. She presents moderate intellectual disability, hypotonia, a characteristic facial appearance, hands typical of CLS, dental anomalies. The rather severe symptoms of the female may be due to the presence of *de novo* one-nucleotide deletion (c.896delT) in exon 11 resulting in loss of important RSK2 domains, as well as observed non-random X-chromosome inactivation.

The study was supported by Polish Ministry of Science Project 0624/P01/2006/31 (N40103131/0624).

#### P01.080

#### MECP2 gene R167W mutation in a girl with autism/Rett-like syndrome and her mildly mentally retarded mother

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Rett syndrome (RTT) is a neurodevelopmental disorder affecting almost exclusively females. The disease is caused by mutations in the X-linked *MECP2* gene, encoding methyl CpG binding protein. Most cases of RTT are sporadic with the majority (99.5%) being caused by *de novo* mutation on the paternal copy of *MECP2*. Familial cases and clinically atypical cases (RTT variants, like preserved speech variant, congenital RTT) show a lower incidence of *MECP2* mutations. Recently *MECP2* mutations in males with severe encephalopathy or mental retardation have been described.

We report identification of *MECP2* gene R167W mutation in a 3 year old girl and her mother.

The girl presents unusual behavioral features: anxiety, emotional and eye contact disturbances, stereotypes (jigging, running, head shaking), sleep and awake breathing rhythm and peripheral circulation disturbances, stretching and paroxysmal cry or clamor. Besides, normal somatic and mental development with preserved speech and voluntary hand use is observed. Mother of the girl, obese woman with development on the edge of normal, presented in her infancy mild psychomotor retardation, hypoactivity, somnolence and lack of criticism. Currently she presents essential tremor and she is still clumsy and talk active. The identified R167W mutation results in substitution of tryptophan in place of arginine-167, situated in the region between MBD and TRD. The mutation occurred on the grandmotherly allele and was *de novo* in the mother. *MECP2* R167W mutation has been previously found in three-generation family with four non-specific X-linked mentally retarded males and unaffected mothers.

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#### P01.081

#### Cerebral anomalies in a case of Xq12q13.1 duplication encompassing the Oligoprenin 1 Gene

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*OPHN1* gene (MIM 300127) mutations are a well established cause of a distinctive phenotype characterized by mental retardation of variable degree, epilepsy, rostral ventricular enlargement and cerebellar hypoplasia. Duplication of this region has never been reported.

We describe the first familial case of a Xq12q13.1 duplication of 800 kb encompassing the *OPHN1* gene, detected by array-CGH. The proband phenotype is characterized by facial dysmorphisms and severe mental retardation associated to specific cerebral anomalies.

Our proband's phenotype never prompted the hypothesis of oligophrenin mutation. This might be due to the specific facial and NMR appearance of *OPHN1* mutated patients (deletions and point mutations). These patients are characterized by long face, wide forehead, deeply set eyes, hypotelorism, long tubular nose, short philtrum and prominent chin. The most striking hallmark shared by *OPHN1* patients on NMR is posterior vermis dysplasia, including lobules VI and VII (de-clive, folium, and tuber) partial agenesis associated with a supplementary right vermian parasagittal cleft and a mild cerebellar hemispheris dysgenesis. Instead our patient showed an altered signal in the so-pratentorial white matter that was more pronounced at the inferior and posterior cerebral hemispheres. Lesions were also described in the pontine tegmentum, corpus callosum, posterior arms of inner capsules and central portions of pallidi nuclei.

To our knowledge this is the first report of a clinical phenotype associated with duplication of Xp12.

#### P01.082

##### Syndromic X-linked mental retardation

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We report a new family with familiar mental retardation and seizures with few dysmorphic facial features.

The proband was born at term after an uncomplicated pregnancy. The boy was the first child from healthy non-consanguineous parents. At the age of 3 years he was referred to medical geneticists for diagnostic advice. The facial appearance was slightly dysmorphic with deeply set eyes, strabismus, a large philtrum, retrognathia and prominent incisors. He had generalised hypotonia, delayed psychomotor development (walking at 3 years), he had no language (he only say few words at 3 years) and significantly mentally retarded. He developed a first epileptic insult during the second year. The metabolic screening and the electroencephalogram were normal. His karyotype was normal, 46,XY.

On examination with 11 years old he had a brain magnetic resonance imaging, it showed large ventricles without hydrocephalus, a large cisterna magna with cerebellar hypoplasia.

He had a maternal uncle and maternal great-uncle with mental retardation and facial appearance similar. His maternal uncle had a test for fragile X negative.

There was a syndromic X-linked mental retardation with cerebellar hypoplasia. We reevaluated the case with 11 years old and analyzed by Multiple Ligation PCR Amplification (MLPA) in the probandus and his mother, but it was not possible in the maternal uncle, with deletion in the exons 3, 4, 5 and 6 of the oligophrenin-1 gene (*OPHN1* gene).

#### P01.083

##### New Rett syndrome and Fragile X syndrome clinical scales for genotype-phenotype correlation studies

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To investigate genotype-phenotype correlations new clinical scales were developed for providing quantitative measurements of 23 Rett syndrome (RTT) and 24 Fragile X syndrome (FXS) phenotypic signs. Scores for separate symptoms summarized to get a total phenotypic severity score.

Correlations between MECP2 mutation type, position and phenotype severity were found in 54 RTT patients. There were significant differences with more severe phenotypic manifestations in patients with truncating mutations compared with those with missense mutations in the phenotype severity scores ( $P<0.001$ , Mann-Whitney test) and phenotypic manifestations scores including hand dyspraxia, impairment of oro-motor functions ( $P<0.01$ ), emotional communication, expressive speech, static functions, walking, head growth and foot size ( $P<0.05$ ). Analysis of phenotype severity dependence on mutation position showed that the most severe phenotypes were observed in patients with R168X, R255X, R270X mutations.

The FXS clinical scale has applied to demonstrate positive correlation ( $R^2=0.7662$ ) between the total phenotype severity scores in 22 patients with FXS and percent of cells containing fragile chromosome X by an approach previously described (Vorsanova et al, 1998).

These results support genotype-phenotype correlations in RTT and FXS and illustrate both scales applicable for estimation of phenotype severity.

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#### P01.084

##### X-linked mental retardation with pachygyria, marfanoid habitus and behavioral problems: a new syndrome?

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X-linked mental retardation (XLMR) is a common cause of inherited mental retardation in males. To date, a total of 215 XLMR conditions have been reported in the literature. We report a 5 generation family segregating an X-linked disorder in which 7 affected males present with distinctive physical features, seizures, mental retardation and behavioral problems. The proband is a 17-year-old man with severe mental retardation, microcephaly, short stature, a marfanoid habitus, seizures, congenital heart defect, pachygyria, irritability, and aggression. Carrier females are physically unremarkable but have aggressive behavior and poor interpersonal skills. Results of laboratory investigations include a normal karyotype, *FMR1* triplet repeat size, serum electrolytes, thyroid function, *MED12* sequencing and biochemical investigations. Linkage analysis localized the disease locus in this family to Xq28. Phenotypically, our proband does not resemble any of the reported XLMR syndromes mapped to this region. Candidate genes in this region are presently being screened by direct sequencing. To our knowledge, the combination of phenotypic features seen in our proband has not been previously reported in any of the XLMR syndromes already mapped to our candidate region.

#### P01.085

##### Xq duplication in a male with mental retardation, facial dysmorphisms, broad thorax, genital anomalies and short stature

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In a male patient with severe mental retardation (MR), a typical facial appearance, a broad thorax, genital anomalies and a disproportionate short stature, a *de novo* Xq13.2q21.1 duplication was identified by array CGH. This 7 Mb duplication encompasses 23 known genes, including the XLMR genes *ATRX* and *SLC16A2*. The phenotype of this patient is similar to the phenotype described in more than ten previously reported cases with overlapping Xq duplications, encompassing MR, short stature, and genital abnormalities comprising cryptorchidism and/or small penis. By re-evaluation of the facial phenotype of these patients and the present patient we observed a similar facial appearance, comprising ptosis, midface hypoplasia with down-slanting palpebral fissures, small mouth, down-turned corners of the mouth and hypotonic facies. The minimal overlapping duplication interval includes the *ATRX* gene, which is known to carry inactivating mutations in patients with alpha-thalassemia/mental retardation syndrome (ATR-X). Detailed comparison of the clinical characteristics and the function of the genes located in the commonly duplicated regions of these patients and the phenotype of animal models with *ATRX* overexpression previously described in literature led us to the hypothesis that an in-

creased dosage of *ATRX* and perhaps other genes are involved in the pathogenic mechanism of this XLMR phenotype.

In conclusion, the association of MR, facial dysmorphisms, broad thorax, genital anomalies, and short stature might contribute to the recognition of male patients with a microduplication encompassing the entire *ATRX* gene. We suggest that male patients with a similar phenotype should be screened for duplications of the *ATRX* gene.

#### P01.086

##### Functional disomy Xqter due to duplication Mecp2 gene

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We report on three patients : 2 boys and a girl, with an additional Xq28 chromosome segment translocated onto 5pter, Ypter, and 13p. The karyotypes were 46,XY,der(5)t(X;5)(q28;pter) 46,X,der(Y)t(X;Y)(q28;pter) and 46,XX,der(13)t(X;13)(q28;p10). In all cases, the de novo cryptic unbalanced X-autosome translocation and Xq-Yp translocation resulted in a Xq28 chromosome functional disomy by duplication of *Mecp2* (methyl-CpG binding protein 2) gene. In all, 20 patients carrying a Xq28 functional disomy could be selected from the literature. Common craniofacial findings include microcephaly, a small mouth. Most patients have prenatal onset growth retardation. Postnatal growth retardation was present in all cases. Major axial hypotonia is constant and usually present at birth. Severe constipation frequently reported. Severe developmental delay is observed in most patients. Functional disomy for the Xq28 chromosome region yields a recognizable phenotype including distinctive facial features, major axial hypotonia, severe feeding difficulties, abnormal genitalia and proneness to infection (pneumonia). Severe developmental delay is almost constant. A clinically oriented FISH study using subtelomeric probes

#### P01.087

##### Functional characterization of the X-linked mental retardation gene ACSL4

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*ACSL4* is a gene involved in non-syndromic X-linked mental retardation. It encodes for a ubiquitous protein that adds Coenzyme-A to long-chain fatty acids, especially arachidonic acid; it presents a brain-specific isoform resulting from alternative splicing and containing 41 additional N-terminal aminoacids. In order to define how *ACSL4* absence causes mental retardation, we have characterized the protein and analyzed the consequences of its absence in neurons. Our data suggest that *ACSL4* is located in endoplasmic reticulum. Quantitative mRNA expression analyses indicate that *ACSL4* is expressed at higher levels in fetal than in adult brain. Moreover, differential expression of the alternative transcripts in different adult brain regions has been observed. Protein analysis has confirmed this variability and revealed a lack of linear correlation between mRNA and protein levels, suggesting the presence of post-transcriptional regulatory mechanisms. To characterize *ACSL4* function in neurons we have silenced the gene by siRNA technology in rat primary hippocampal neurons. Our data suggest that *ACSL4* might be important for dendritic spine formation and/or maintenance. In fact, *ACSL4* absence seems to cause a significant reduction in dendritic spine density and alteration in spine distribution among different morphological categories. Moreover, abnormal actin accumulation has been observed in a significant percentage of cells. This last finding suggests that *ACSL4* might directly or indirectly influence actin cytoskeleton organization; it could be thus hypothesized that the observed spine anomalies are a secondary effect of an abnormal actin organization due to *ACSL4* absence. Additional experiments will be necessary in order to confirm these hypotheses.

#### P01.088

##### Molecular characterization by aCGH of a 3,8 Mb duplication at Xq26.3 in a male with mental retardation

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Males with duplications in the distal long arm of the X chromosome are rare and in most cases are inherited from a carrier phenotypically normal. We report the clinical and molecular characterization of a Xq26.3 duplication in a male and his brother affected by MR. Chromosome analysis was normal and Multiplex Ligation Probe Amplification (MLPA) analysis detected a duplication of the *ARHGEF6* gene inherited from a carrier mother. Both affected brothers presented moderate mental retardation and displayed dysmorphic features. Further characterization of the duplication by array CGH and FISH experiments with specific BAC probes, revealed a deletion of 28 contiguous BAC clones, spanning a region of 3,8 Mb in Xq26.3. X-inactivation studies in the mother showed a complete skewed X-inactivation (100/0) inactivating the X-chromosome inherited by the patient. Among the 20 genes included within the duplicated region we discuss the implication of *ARHGEF6*, *PHF6* and *HPRT1* in the phenotype of the patient. Mutations or deletions in these three genes are responsible for syndromic and non-syndromic forms of mental retardation. Nowadays high-resolution technologies such as array CGH allow the detection of copy number aberrations in patients with MR. The characterization of these cryptic rearrangements is of clinical importance in order to provide a genetic counselling in carrier women for future pregnancies.

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#### P01.089

##### Fragile X syndrome and Xp deletion in a girl with autism and mental retardation

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Autism is a complex behavioural disorder characterised by social and communication impairments, and restricted repetitive and stereotyped behaviour. It is often associated with mental retardation. Genetic factors play an important role in the aetiology of autism - heritability reaches 90 %, one of the highest among psychiatric disorders. The inheritance is most likely multifactorial with genetic heterogeneity and complex interactions. Despite of all efforts, until now the success in finding of autism susceptibility genes has been limited. Cytogenetic abnormalities and single-gene defects often associated with autism (e.g. the fragile X syndrome) together account for about 10% of cases. However, these are the only cases where diagnostics and exact risk assessment are possible. We present a 7-year-old girl with atypical autism, mental retardation, hyperactivity, developmental delay, facial dysmorphism and overweight. Her family showed no history of mental retardation or autism, but tremor was apparent from sixty years of age in her maternal grandfather. DNA testing of the patient revealed full mutation (460 CGG repeats) in the *FMR1* gene, consistent with the diagnosis of fragile X syndrome. In addition, cytogenetic analysis detected a large deletion on chromosome Xp. We attempted to define in more detail the breakpoints of the deletion and the inheritance of both genetic defects in the family. Microarray CGH mapped the 17.5 Mb deletion to Xp22.11-p22.31. The deleted segment harboured almost 100 protein-coding genes. The distal breakpoint of the deletion was located close to *NLGN4*, a gene implicated in autism and mental retardation. Supported by grants NR/9457-3 and MZO00064203

**P01.090****Fragile X mosaic male detected by PCR/MS-MLPA**

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We report on a fragile X syndrome (FXS) mosaic male full mutation/normal allele, detected by a combination of PCR and methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). This analysis provides a powerful, fast, cheap and easy to perform diagnostic approach for FXS. This is the first report on successful application of MS-MLPA for FXS diagnostic purposes. The combination of PCR and MS-MLPA gives the possibility in a few steps to detect normal FMR1 alleles, to prognose the expanded ones, to assess the CpG islands methylation, as well as to determine copy number changes like large deletions/duplications, not only along the *FMR1*, but also along the *FMR2* gene.

Our PCR results showed one allele of 29±1 repeats in the mother and one allele in the affected boy, but three repeats larger - 32±1 repeats. The MS-MLPA results in the patient showed hypermethylated full mutation pattern in comparison to the normal control. The MS-MLPA data calculations were performed in Excel.

In our opinion, the mosaic pattern of normal size/full mutation alleles was a result from inheritance of a maternal unstable premutated allele. Most logical mechanism for normal size allele generation in our mosaic case is a deletion of a portion of the full mutation, restricted to the CGG repeat itself, as the primers for PCR were designed in the repeat flanking regions. The reported patient demonstrates atypical mild clinical manifestation of the disease, which might be due to the presence of a normal size allele in a large percentage of the patient's cells.

**P01.091****No evidence for skewed X-inactivation in fragile X syndrome premutation carriers**

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X-Chromosome inactivation (XCI) is the mechanism by which gene dosage equivalence is achieved between female (XX) and male (XY) mammals. In the general female population, the X-inactivation process is random, and therefore XCI ratios have a normal distribution (with average of 50:50) with only a small percentage of females (5-8%) showing a skewed X-inactivation ratio (>90:10). Fragile X syndrome (FXS) premutation carriers (55-200 CGG repeats) do not present FXS symptoms but it has been shown that they have a higher risk of developing premature ovarian failure (POF) and/or fragile X associated tremor/ataxia syndrome (FXTAS). About 20% of the FXS female premutation carriers present POF and around 15% FXTAS. In order to evaluate if these pathologies are associated with skewed XCI patterns, we have studied the X-inactivation pattern in 270 FXS females carriers (41 POF, 2 FXTAS, 3 POF and FXTAS, and 224 no POF no FXTAS). Results showed that FXS permutation female carriers have a normal distribution and that there is no correlation with the CGG repeat number. On the basis of these observations we conclude that FXS female premutation carriers with or without POF and/or FXTAS do not present skewed X-inactivation and therefore, other molecular or environmental factors may predispose to these conditions.

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**P01.092****High conservation of the 3'UTR of *FMR1* at potential microRNA target sites in patients with fragile X syndrome premutation**

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Fragile X syndrome premutation condition consists of an intermediate CGG repeat expansion (55-200 repeats) at the 5'UTR of the *FMR1* gene. In the premutation range, *FMR1* mRNA levels are increased

whereas the amounts of FMRP are slightly reduced compared with the control population, suggesting that posttranscriptional regulation of *FMR1* could be involved in these disorders. MicroRNAs act as regulators of gene expression binding to their target sites located at the 3'UTR in a high number of protein-coding genes inducing cleavage or repression of translation. We have screened the 3'UTR of *FMR1* in 40 Mediterranean premutation carriers and 14 control subjects with no expansion in the *FMR1* gene. Overall, the *FMR1* 3'UTR region appears as a high conserved region, not only in humans, but also in other species. Our study excludes changes in the *FMR1* 3'UTR in the premutated patients, ruling out a possible role of microRNA target sites in *FMR1* regulation in permuted phenotypes. Acknowledgements (SAF2004-03083, Marató TV06-0810)

**P01.093****Molecular and epigenetic characterization of *FMR1* unmethylated full mutation cell lines**

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Fragile X syndrome (FXS) is mostly caused by expansion and subsequent methylation of the CGG repeat at the 5' UTR of the *FMR1* gene (methylated full mutation). Individuals of normal intelligence, carriers of a *FMR1* unmethylated full mutation, represent rare exceptions. We previously performed a molecular and epigenetic analysis of a lymphoblastoid cell line (code 5106), derived from one of these individuals. Recently, two apparently normal individuals with an unmethylated full mutation, belonging to distinct FXS families, were identified. From each subject (named DPM and MA) three independent lymphoblastoid cell lines and a fibroblast culture (from MA) were established. In accordance with our previous findings, these cell lines showed normal transcription and reduced translation of the *FMR1* gene, compared to normal controls. Epigenetic analysis of the *FMR1* locus demonstrated lack of DNA methylation and the methylation pattern of lysines 4 and 27 on histone H3 was also similar to that of a normal control, in accordance with the normal transcription and consistent with an euchromatic configuration. On the other hand, the H3 and H4 acetylation and the methylation of lysine 9 on histone H3 was similar to that of a typical FXS cell line. Comparative analysis of these rare unmethylated full mutation cell lines demonstrates remarkable structural, functional and epigenetic consistency, suggesting a common mechanism of origin, genetically determined. The discovery of such mechanism may be important in view of therapeutic attempts to convert a methylated to unmethylated full mutation, restoring the expression of the *FMR1* gene.

**P01.094****Analysis of fragile-X premutation and grey zone in paediatric patients**

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INTRODUCTION: The number of CGG repeats defines the state of premutation or full mutation in fragile-X syndrome (FXS). In childhood, full mutation has a characteristic behaviour and physical phenotype. The adult carriers of the premutation can develop a progressive neurodegenerative syndrome (FXTAS). Although the clinical characteristics of the premutation and grey-zone in the childhood are less well-known, clinical experience and case reports suggest that child with premutation and grey-zone alleles may have similar clinical features than those with the full mutation.

PATIENTS AND METHODS: Determination of the number of CGG repeats in samples received in our unit since 2005 for the FXS screening.

Detailed Clinical and cognitive evaluation in a series of 13 children (9 boys and 4 girls), born between 1989 and 2000, with clinical suspect of FXS.

RESULTS: From the 1092 samples analyzed, 7% (77 cases) we detected alleles with 35 to 160 CGG repeats. We haven't detected any alleles in the permuted or grey-zone in the 91 control chromosomes analyzed. The 13 children evaluated clinically have a cognitive-behavioural phenotype suggestive of full mutation and we detected alleles

between 35 to 53 repeats. Clinical findings in this group of patients will be commented.

**CONCLUSIONS:** We have detected a big number of alleles in the pre-mutation and grey zone in the population analyzed. Large independent samples are required to confirm our impression that the grey-zone and premutation in FXS may be associated with important developmental disabilities and to establish the genotype-phenotype relation in children with this range of repeats.

#### P01.095

#### Is FRAXE over-represented among Newfoundland children with cognitive impairment?

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Fragile X syndrome due to mutations in the *FMR1* gene is the commonest cause of inherited mental retardation. In contrast, mutations in *FMR2* (FRAXE) are a much rarer cause of MR. In Newfoundland, only one family segregating an *FMR1* expansion has been identified, but 3 males from 2 families with FRAXE disease have been identified. In this study, I reviewed the charts of all boys seen from 1994-2004 who had negative *FMR1* testing. Participants were recruited using the following criteria - males with developmental delay, between the ages of 2 and 19 and without a specific diagnosis. 139 of 378 individuals met the inclusion criteria, 95 (68%) consented. DNA was collected and amplified using previous published primers. 93 samples were in the normal FRAXE range, 2 failed to amplify. These 2 samples are being further studied as possible FRAXE mutations. FRAXE alleles ranged from 4 to 23 repeats with the most common allele size being 13. A second part of the study describes the previously identified FRAXE families: One proband has an *FMR2* expansion of 620 repeats. He has a FSIQ of 54 and is dysmorphic with facial features reminiscent of FRAXA syndrome. He has a non-dysmorphic first cousin with a learning disability and an expansion of 200 repeats. His mother and maternal aunt carry premutations of 120 and 87 repeats respectively. The second proband is a non-dysmorphic 11-year-old with attention deficit disorder and a FSIQ in the 80's. He has a mosaic *FMR2* expansion of 120-820 repeats.

#### P01.096

#### Homozygous R316Q mutation in obesity-associated FTO gene causes a novel polyomformative syndrome

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We report on the analysis of a large inbred family including 8 affected children presenting a newly described syndrome characterized by intra-uterine growth retardation, characteristic facies, cleft palate, cardiac and genital abnormalities, central nervous defects with delayed myelination, severe hypertonicity and premature death. Extensive workup was normal.

Since the pedigree suggested an autosomal recessive mode of inheritance, autozygosity mapping was performed identifying a unique region of shared homozygosity of 6.5 Mb on 16q12 between D16S411 and D16S3140 markers. We identified a homozygous missense mutation within FTO gene (c.947G>A, p.R316Q), which altered a highly conserved residue, co-segregated with the disease and was not found in 400 control alleles.

While recent studies have revealed a strong association between intronic variants in FTO gene and childhood and adult obesity, the pathophysiological mechanism underlying the phenotype observed in our family remains questionable. Noticeably, *in-situ* hybridization experiments on human embryos showed predominant FTO expression in tissues affected in patients (central nervous system, heart and frontonasal prominence).

Interestingly, a recent study showed that FTO encodes a 2-oxoglutarate-dependent nucleic acid demethylase likely involved in toxic DNA and/or RNA lesions repair. Moreover, a R316A substitution completely abolishes Fto activity *in-vitro*. We consequently propose that the patients' mutation is a null mutation and that their phenotype is the consequence of defective DNA repair. Mass spectrometry assays and cellular sensitivity to methylating agents measurements are underway to

validate this hypothesis.

In conclusion, our data suggest that homozygous FTO null mutations are responsible for a hitherto undescribed multiple congenital anomaly syndrome.

#### P01.097

#### Studies on *FMR1* gene premutations, causing Fragile X tremor ataxia syndrome (FXTAS) in Polish ataxia patients of unknown etiology and in controls

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Fragile X tremor ataxia syndrome (FXTAS) is a late onset ( $\geq 50$ ) syndrome, observed generally in male patients, caused by pre-mutation (range 55 - 200 CGG) in *FMR1* gene (Hagerman 2001). The main symptoms are: intention tremor, ataxia, parkinsonism, and cognitive decline. Moreover the MRI shows the presence of white matter lesions of the middle cerebellar ppenducles.

The aim of our study was to look for FXTAS in a large group of Polish patients affected with ataxia of unknown origin and in controls.

The studied group comprised 176 male patients with sporadic cerebellar ataxia, with/without other neurological symptoms (age of onset  $\geq 50$  years), in whom molecular tests carried out previously, excluded SCA1,2,3,6,7,8,12,17 and DRPLA.

The group of controls was composed of 516 healthy subjects. The number of CGG repeats was determined by comparison of PCR product size with size standard after electrophoresis on ABI PRISM377 in 4% denaturing gel.

In the ataxia group we found no pre-mutation alleles, and the largest allele found contained 52 CGG repeats.

The *FMR1* normal allele obtained for the control group ranged from 14 to 56 CGG repeats, with the most frequent 29 CGG alleles.

These results indicate that Polish control group is characterized by similar *FMR1* gene polymorphism as other populations. And since pre-mutation in the patients group was not found, we think that FXTAS among patients with sporadic ataxia is less frequent than we expected; we plan to enlarge the studied group by including patients with other neurological symptoms, such as parkinsonism.

#### P01.098

#### Frequency of fragile X tremor ataxia syndrome in fragile X syndrome families

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Fragile X syndrome (FXS), which is caused by a CGG triplet expansion in the first exon of *FMR1* gene, is the leading cause of familial mental retardation. Premutated individuals (55-200 CGG) do not present FXS symptoms, but approximately one third of them can present other manifestations such as a late onset ataxia /tremor syndrome (called FXTAS) or premature ovarian failure (POF). In order to determine the presence of FXTAS among FXS grandparents, we have contacted by telephone with 92 families. We have identified 24 premutated carriers (belonging to 18 FXS families) showing FXTAS symptoms. These results evidence that FXTAS frequency among FXS families is around 20%. The youngest individual is 66 and the oldest 82 y.o. We are now evaluating these patients psychologically, neurological and with magnetic resonance imaging. Molecular studies confirm a slight reduction of FMRP protein and increased levels of mRNA in these patients (x2-x5 folds). The description of associated pathologies (FXTAS and POF) to premutation carriers has modified genetic counseling for FXS, these two disorders and their consequences have to be taken into account by genetic counselor.

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**P01.099****Deletion of MAOA and MAOB only in a male patient causes severe developmental delay, intermittent hypotonia and stereotyped hand movements.**

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The monoamine oxidases MAOA and MAOB are isoenzymes which catalyse the oxidative deamination of biogenic amines, with substrates including the neurotransmitters norepinephrine, serotonin and dopamine. A number of behavioural traits, such as aggressive and antisocial behaviour, and psychiatric conditions, such as schizophrenia and bipolar disorder, have been associated with MAOA/B dysfunction and genetic variation at these loci.

The *MAOA* and *MAOB* genes occur in tandem but in opposite orientations on Xp11.23. The two genes share 70% identity at the amino acid level and exhibit identical organisation of their 15 exons. Previous reports of *MAOA* and *MAOB* deletions have encompassed the adjacent *NDP* gene. Mutations in *NDP* cause Norrie disease, a disorder characterised by blindness, progressive sensorineural deafness and, frequently, mental retardation. Genotype-phenotype correlations in Norrie disease suggest that deletions of *NDP* which also extend to the *MAOA/B* genes result in a more severe neurological phenotype. However, the phenotypic contribution of *MAOA/B* gene disruption is difficult to dissect in this context. Here we report an individual with a submicroscopic deletion which encompasses only the *MAOA* and *MAOB* genes. The 246kb deletion, which includes exons 2-15 of *MAOA* and the entire coding region of *MAOB*, was detected by high-resolution long oligonucleotide X chromosome array comparative genomic hybridisation, and confirmed by fluorescence in situ hybridisation. The phenotype of the affected boy was severe mental retardation with unusual hand posturing. The obligate mother of the affected boy was clinically normal.

**P01.100****Raised T3 levels and mutations in *MCT8*(*SLC16A2*) cause X-linked cerebral palsy and mental retardation**

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Mutations or whole gene deletions of *MCT8* (*SLC16A2*) were identified in males with raised serum T3 concentrations and severe psychomotor retardation. The associated phenotype prompted us to screen *MCT8*(*SLC16A2*) in males with X-linked mental retardation with spastic quadriplegia and those with XLMR alone. We have identified 3 mutations ( 1018delC, A224T,R271H) and 2 single exon deletions (exon 1 and exon 6) in families with X-linked spastic quadriplegia and severe mental retardation but did not find any mutations in >300 families where mental retardation alone was the presenting feature.

Prior to identification of the mutation all cases were all described as severe cerebral palsy. Males present with severe hypotonia at birth and develop a severe spastic quadraparesis in all cases within the first year of life that remained static once it had evolved. Brain MRI in one affected individual was normal. None of the affected individuals were able to walk independently or had intelligible speech and all needed full-time care either in institutions or at home. Difficulties with weight gain and difficulty feeding are consistent features. Thyroid hormone profiles were performed in all families once sequence variants in the *MCT8*(*SLC16A2*) gene were identified. Carrier females were found to have free T3 levels at the upper limit of normal between 7.0 and 7.5 (3.0-7.5) pmol/l whereas the affected males were found to have raised T3 levels 9.6 - 13.7 (3.0-7.5) pmol/l. Free T4 and TSH levels were within the normal range in all affected males and carrier females.

**P01.101****Duplications of the MECP2 gene region and severe mental retardation in males**

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Mutations in the *MECP2* gene are associated with Rett syndrome in females and a spectrum of neurological disorders in males, from mild mental retardation to severe neonatal encephalopathy. More recently, duplications of the *MECP2* gene have been described in males with severe mental retardation and a range of other progressive neurological symptoms. Using the multiplex ligation-dependent probe amplification (MLPA) assay, we have screened 20 male patients who have been specifically referred for *MECP2* gene duplication analysis, with symptoms including severe mental retardation, epilepsy and an "Angelman like" phenotype. Duplications involving the *MECP2* gene were detected in five cases (25%), four of these being in unrelated males and one in a similarly affected brother. Two of the unrelated cases show complex rearrangements of the *MECP2* gene. These results and others that have been published recently, show rearrangement of the *MECP2* gene to be a relatively common mutation mechanism in males. This redefines the testing criteria for referral for *MECP2* analysis. We will attempt to further determine the clinical phenotype for males with *MECP2* gene duplications, to help improve service provision.

**P01.102****A Study on the Most Common Genes Involvement among Iranian Families Who Have RETT Syndrome**

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*MECP2*-related disorders include classic Rett syndrome, variant or atypical Rett syndrome, and mild learning disabilities in females and neonatal encephalopathy and mental retardation syndromes in males. Classic Rett syndrome is a progressive neurologic disorder in girls characterized by normal birth and apparently normal psychomotor development during the first six to 18 months of life. The girls then enter a short period of developmental stagnation followed by rapid regression in language and motor skills. Seizures occur in up to 90% of affected females; generalized tonic-clonic seizures and partial complex seizures are the most common. Females with classic Rett syndrome typically survive into adulthood, but the incidence of sudden, unexplained death is significantly higher than in controls of similar age. Atypical Rett syndrome is increasingly observed as *MECP2* mutations have been identified in individuals previously diagnosed with autism, mild learning disability, clinically suspected but molecularly unconfirmed Angelman syndrome, or mental retardation with spasticity or tremor.

Rett syndrome is inherited in an X-linked dominant manner. Approximately 99.5% of cases are single occurrences in a family, resulting either from a *de novo* mutation in the child with Rett syndrome or from inheritance of the disease-causing mutation from one parent who has somatic or germ line mosaicism.

PCR amplification and Sequencing of the three exons of *MECP2* gene coding region showed that R 407 Stop codon, R 224C and A 72 R were heterozygote mutations in all Iranian female patients.

**P01.103****X-linked mental retardation(XLMR) and the *MECP2* gene**

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*MECP2* is an X-linked gene encoding a nuclear protein that binds specifically to methylated DNA. Until recently the gene was predicted to function as a general transcriptional repressor. An alternative function however has been recently reported indicating the primary function of *MeCP2* not being the silencing of methylated promoters.

Mutations in *MECP2* have been reported to be the molecular basis of a broad spectrum of neurological disorders including : Rett syndrome (RTT), unexplained progressive encephalopathy and an Angelman-like phenotype. Also, duplications of *MECP2* region are associated with 10% of familial X-linked mental retardation (XLMR). The XLMR phenotypic spectrum often includes hypotonia, spasticity, absent speech and recurrent infections.

To date the Division of Human genetics, University of Cape Town has

DNA banked from 80 XLMR families and 30 male sib-ships that are negative for mutations in the most common XLMR associated genes (*FMR1* CGG expansion and *ARX* mutations). This group of patients was stratified into a smaller cohort of subjects according to the clinical criteria indicated above. Preliminary findings involving qPCR analyses, suggests significant *MECP2* involvement in XLMR patient phenotypes.

This study further assesses the degree of *MECP2* involvement in an XLMR cohort of South African patients using MLPA analysis of the *MECP2* chromosomal region in a larger group of XLMR patients. Determining the proportion of XLMR patients in the Human Genetics bank, due to *MECP2* mutations will assist in the genetic management of XLMR families through definitive diagnostic systems, carrier ascertainment and prenatal diagnoses to carrier females.

#### P01.104

##### **MECP2 gene mutation analysis in patients with Rett-like features in Latvia**

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Rett syndrome (RTT), an X-linked dominant neurodevelopmental disorder mostly in females, with an incidence of 1 in 10 000-15 000 female births. Gene *MECP2* (methyl-CpG-binding protein) gene has been identified as the disease-causing. RTT is one of the most common causes of mental retardation in females. After a period of normal development usually until 6-18 months of age, affected girls enter a period of regression, losing speech and motor skills, coincident with the onset of hand stereotypies, leading to loss of purposeful hand use, which is the hallmark of the disorder.

*MECP2* gene is located on chromosome Xq28 and is subject to X-inactivation. Gene mutations are identifiable in 80% of classic Rett syndrome, but less frequently in atypical RTT.

First 10 unrelated patients (including 2 boys) with developmental delay and autistic features were referred for molecular diagnostic. Genomic DNA was extracted from blood leukocytes. *MECP2* coding exons 2, 3, and partly exon 4, were amplified in 7 overlapping PCR fragments and analyzed by direct sequencing on ABI 310 genetic analyzer. No previously reported mutations were found in analyzed fragments. Studies of remaining part of *MECP2* gene exon 4 for most common mutations in TRD domain are in progress. Additional mutation analysis of exon 1 will be performed after exons 2, 3 and 4 complete investigation. Novel changes found in *MECP2* gene exon 4 should be confirmed by restriction analysis.

#### P01.105

##### **Analysis of polymorphisms in 5-HTT, HSP 70-1, APOE, and HMOX-1 genes as potential modulation factors of Rett syndrome phenotype**

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**Background:** Rett syndrome is a severe X-linked neurodevelopmental disorder caused primarily by de novo mutations in the *MECP2* gene. It is one of the leading causes of mental retardation in females with prevalence 1:10,000 female births worldwide. The phenotypic spectrum of Rett syndrome is very variable even in females with random X chromosome inactivation. Therefore we hypothesize there might be other genetic factors which modulate the Rett phenotype. In a large case-control study, we performed molecular genetic analysis of functional polymorphisms in several genes involved in neuronal development and metabolism (5-HTT, HSP70-1, APOE, and HMOX-1).

**Methods:** All patients carried confirmed pathogenic mutation in the *MECP2* gene. Molecular genetic analysis of six polymorphisms was performed using PCR-based methods (PAGE, PCR/RFLP). Results were statistically evaluated by the test of binomial distribution.

**Results:** Our findings revealed a statistically significant difference between patients and controls for allele and/or genotype distribution of *APOE*, 5-HTT, and *HSP70-1* (-110A>C), but not for associations between Rett syndrome and polymorphisms (GT)n in the *HMOX-1* promoter or +190C>G in *HSP70-1*.

**Conclusion:** To our knowledge this is the first such study in Rett syndrome patients and further confirmation in experimental and epidemiological studies is necessary. Understanding the inherited factors that influence patients' susceptibility for developing various Rett phenotypes may lead to the development of better and more comprehensive therapies.

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#### P01.106

##### **CDKL5 gene mutations in patients with a RTT-like phenotype**

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Rett Syndrome (RTT) is a progressive neurodevelopmental disorder showing several phenotypic manifestations. The majority of clinically diagnosed cases have been found to show a mutation in the *MECP2* X-linked gene, a gene that encodes for a transcription suppressor Methyl-CpG-Binding Protein. The cyclin-dependent kinase-like 5 gene (CDKL5) is another X-linked gene that belongs to the same molecular pathway of *MECP2* and encodes a phosphorylated protein with protein kinase activity. Mutations in the CDKL5 gene cause severe mental retardation, early onset epilepsy and drug resistance.

We have screened the 21 exons of the CDKL5 for mutations in 18 female patients with a RTT-like phenotype and epilepsy who had tested negative for *MECP2* mutations. The aim of this study was to determine whether the condition of these patients is due to mutations in the CDKL5 gene. Genomic DNA was extracted using standard procedures from the peripheral blood leukocytes of patients. Mutation analyses were performed using CSGE in 24 fragments of the CDKL5 gene and sequenced in case of anomalous bands.

We have only found 2 cases with variations: three nucleotide exchanges that form a rare conserved haplotype - IVS4+17A>G, c.3003C>T (H1001H) and c.3084G>A (T1028T) (J Tao et al. 2004) - in one patient and a new missense change in the 17<sup>th</sup> exon - c.2389G>A (D797N) - in another patient without pathologic effect because the father was the carrier.

These results indicate that mutations in the CDKL5 gene are not an important contribution in the ethiology of RTT with epilepsy in our population.

#### P01.107

##### **Searching for Copy Number Polymorphisms as modifiers in RTT syndrome**

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*MECP2* mutations are associated with a broad spectrum of clinical phenotypes in girls, including the preserved speech variant (PSV) of RTT. In this variant, girls improve language and motor abilities. Previous studies demonstrated that mutation type and/or X chromosome inactivation are not sufficient to explain such variability, suggesting that additional factors are involved. We hypothesized that Copy Number Polymorphisms (CNPs) contribute to RTT clinical variability. We started to search such variations in three familial cases with the same *MECP2* mutation and different phenotype (<http://www.biobank.unisi.it>): two pairs of sisters (one classic and the other PSV) and a mother/daughter pair (mother with mental retardation and daughter with classic RTT). In these cases, we performed whole genome array-CGH and we found a total of 18 CNPs. Three of them (6p21.33, 8p11.23, 14q11) are in common between two familial cases. Interestingly, the 8p11.23 region includes ADAM5. ADAMs are transmembrane proteins that play an important role in the development of the nervous system. They regulate proliferation, migration, differentiation and survival of various cells, as well as axonal growth and myelination. Among the

other CNPs, the 1q42.12 region contains the *ENAH* gene that represents a good candidate as RTT modifier since it is involved in the pathways that control cortical neuronal positioning. Real-time qPCR with specific probes for selected candidate genes in 100 classic and 20 PSV patients is ongoing. The identification of modifier genes will allow to better characterize the pathogenic mechanisms of RTT, giving additional handles for therapy design.

#### P01.108

##### Rett syndrome in two years old girl with Xp deletion - case report

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Rett syndrome (RTT; OMIM#312750) is an early childhood neurodevelopmental disorder. A girl at age of 2 years was referred to our laboratory for DNA analysis of Rett syndrome. She demonstrated some of the symptoms, characteristic for Rett syndrome - arrested mental development, loss of communication skills, speech delay and purposeful hand movements, appearance of autistic features. Under some circumstances the child demonstrates aggressive behavior.

The performed cytogenetic analysis showed pathological karyotype - 46,XX,del(X)(p1.22).

About 85% of the cases with Rett syndrome were caused by mutations in the gene encoding methyl-CpG-binding protein 1 (MECP2). In about 10% of the cases the disease causing mutation affects the gene for cyclin dependent kinase like 5 (CDKL5). However, the causative mutation is still unknown in the remaining 5% of the cases.

The complete sequencing of MECP2 and CDKL5 genes revealed no mutations in the affected girl. In addition we sequenced the Aristaless related homeobox (ARX) gene, mutations in which have been shown to cause mental retardation either isolated or associated with a broad spectrum of neurological problems. This gene has been mainly affected in boys. As this gene is localized on the short arm of the X-chromosome (Xp22.13), one of its copies was missing in our Xp deleted girl. For that reason this gene seemed to be a good candidate for screening in our mentally retarded girl. The DNA analysis of ARX showed no pathological changes.

We will appreciate any further comments and suggestions on the reported case.

#### P01.109

##### ARX mutations in South African patients with X-Linked mental retardation (XLMR): Research to diagnostics

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**Objectives:** Mental retardation (MR) is associated with decreased cognitive function and impaired development of adaptive skills. The genetic heterogeneity of the disorder is exemplified by the number of genes implicated in the pathogenesis of MR. Of these genes, a substantial proportion are located on the X chromosome, giving rise to the term X-linked mental retardation (XLMR). Mutations in the ARX gene are the second largest contributor to XLMR, preceded only by CGG expansion mutation in *FMR1*, responsible for Fragile X syndrome.

**Methods:** DNA sequence alterations in the ARX gene were investigated in 119 XLMR patients, 32 patients that form part of a male sibship and 183 isolated cases (all individuals are *FMR1* expansion mutation negative). These analyses were conducted using denaturing high-performance liquid chromatography (dHPLC), or PCR amplification and gel electrophoresis of the common c.428\_451dup (Dup24) mutation alone.

**Results:** To date the proportion of ARX disease-causing mutations in the XLMR cohort is 2.4% and 3.4% in the sib-ships. While in the isolated group the Dup24 mutation has not been detected.

**Conclusion:** These findings suggest that c.428\_451dup testing is feasible and justified in determining the cause of XLMR in males negative for Fragile X syndrome, in the Western Cape region of South Africa. Incorporating this test into the diagnostic protocol stands to improve the diagnostic yield of patients, which in turn will afford improved genetic management of families. Furthermore, better genetic management will have a valuable impact on the burden of disease in a developing country such as South Africa.

#### P01.110

##### Molecular diagnostic of XLMR in mentally retarded males from Latvia

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Mental retardation (MR) is one of the main reasons for referral in paediatric, child neurological and clinical genetic practice. The prevalence of mental retardation is thought to be on the order of 2-3%.

X-linked gene defects have long been considered to be important causes of mental retardation, on the basis of the observation that mental retardation is significantly more common in males than in females. Clinical observations and linkage studies in families revealed that X-linked mental retardation (XLMR) is a highly heterogeneous condition. The most common form of 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 group of 341 unrelated males with MR referred from clinical geneticists was screened for FXS. CGG repeats number was detected by Applied Biosystems protocol on ABI Prism 310. The prevalence of 29, 30 and 31 CGG repeats for normal alleles were found. Six affected patients were detected (1.76%). The final diagnosis of FXS was confirmed by Southern blotting. DNA sequencing for the estimation of AGG inserts structure for gray zone (34-50 repeats) alleles was used.

To analyse undiagnosed XLMR patients we recently introduced the multiplex ligation-dependent probe amplification (MLPA) method to identify deletions and duplications of MRX genes. We use MLPA P106 MRX commercial kit (MRC-Holland) to analyse one or more exons of 14 MRX genes.

#### P01.111

##### Novel PCR-based protocol for a rapid molecular testing of Fragile X syndrome

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Fragile-X syndrome (FXS) is the most common cause of inherited mental retardation. It is caused by an anomalous expansion of CGG repeats in the 5'UTR of the *FMR1* gene, which are hypermethylated, causing the absence of *FMR1* expression.

Clinical diagnosis of typical FXS is usually difficult to establish before puberty and molecular testing is therefore needed to confirm it.

Several PCR protocols have already been developed to amplify these repeats, but neither of them is able to amplify full-mutated alleles.

With a view to improving the molecular analysis of FXS, we have established a new PCR-based strategy.

We analyzed 68 retrospective samples of known repeat sizes to assess protocol efficiency and 252 prospective samples.

Two PCRs were performed followed by electrophoresis in a DNA sequencer. Finally, a methylation-specific PCR was carried out to test the promoter methylation status.

Results from retrospective samples were reproduced with the new protocol, supporting its capability to test the whole range of *FMR1* alleles. Additionally, its sizing accuracy made allele frequency distribution and transmission studies possible.

Analysis of prospective samples revealed 13 full mutations, two pre-mutation-full mutation mosaisms, 6 premutations and 10 grey-zone patients.

Moreover, we have amplified and accurately sized a full-mutated allele of 817 repeats, the longest allele amplified by PCR until now.

In conclusion, this PCR approach has improved and speeded up the FXS diagnosis making it less labor-intensive than standard procedures that include Southern blot analysis.

**P01.112****Evaluation of a PCR-based method that allows the analysis of fragile X mutations in the complete range of expansions: a multicentric Italian experience**

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We present the results of a collaborative study aimed at evaluating the performances of the Fragile X PCR Assay (Abbot Molecular, Inc), designed to provide robust amplification and precise sizing of FMR1 dynamic mutations within the entire range of expansions. The goal of the study was to test the following features: 1) accuracy in measuring the number of CGG repeats; 2) capacity to resolve normal FMR1 alleles differing by one triplet unit, 3) capacity to assure efficient co-amplification of different-size fragments; 4) amplification of full mutations. The population studied comprised a total of 378 individuals, 241 females and 137 males, all previously ascertained by Southern blot and other types of PCR-based analysis which found 226 individuals with wt FMR1 alleles, 80 with premutations, 48 with full mutations and 24 with complex mosaicism. A few representative samples were analyzed by all the laboratories involved. The study pointed out the importance of evaluating the whole set of data generated through the analysis for a correct interpretation of the final results. The work confirmed the sensitivity and reliability of the method that allowed to correctly size FMR1 alleles in the different categories of expansions and obtained a robust amplification of full mutations with up to 600 repeats. The Fragile X PCR Assay is a molecular tool that can fill the existing gap between currently used PCR-based methods that do not amplify large CGG expansions and Southern blot analysis which does not allow a precise sizing of FMR1 alleles.

**P01.113****Tissue mosaicism of unmethylated expanded FMR1 allele derived from normal number of CGG repeats**

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Fragile-X syndrome (FRAXA, OMIM #300624) is caused by expansion of a [CGG] trinucleotide in the FMR1 gene over 200-230 repeats. This leads to methylation of CpG islands of its promoter region, which in turn causes silencing of FMR1 gene. Length and methylation mosaisms are described, arisen from premutated or mutated maternal alleles, with different and often unpredictable clinical outcome. Here we present the molecular characterization of a clinically normal 33 years old man referred to us for genetic counseling after the identification of a carrier status (61 [CGG]) of her daughter detected during amniocentesis and confirmed at birth. Familial study revealed a maternal transmission of the premutated allele. Surprisingly, a mosaic pattern ranging from normal to 330 unmethylated FMR1 alleles was detected in the father. On clinical re-evaluation, no sign associated with FRAXA were detected, except relative macroorchidia and slightly extroverted ears. Intelligence were above-average. Samples of fibroblasts, sperm and saliva were additionally analyzed and all resulted normal. QF-PCR and chromosome analysis excluded additional chromosome X mosaicism. Blood sample of his mother showed the same normal allele, as resulted from linkage analysis. To our knowledge, even if tissue-confined, this is the first report of expanded FMR1 allele originating from normal allele.

**P01.114****Variation in novel exons (RACEfrags) and human genetic disorders: the case of Rett syndrome**

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The study of transcription using genomic tiling arrays has lead to the identification of numerous additional exons. One example is the MECP2 gene on the X-chromosome; using 5'RACE and RT-PCR in human tissues and cell lines, we have found more than 15 novel exons (RACEfrags) connecting to at least one exon of MECP2 gene and map up to 1 Mb telomeric to it. We subsequently asked if variation in the novel exons is causatively associated with Rett syndrome. We sequenced all MECP2-connected exons and flanking sequences in 3 groups: 48 Rett patients without mutations in MECP2 and CDKL5 genes (group\_1); 30 Rett patients with mutations in the MECP2 gene (group\_2); 100 control individuals from the same geoethnic group (group\_3). Approximately 14kb was sequenced per sample, (2.6Mb of DNA resequencing). 75 individuals had new, not yet identified rare variants, but no statistically significant difference was found among the 3 groups. These results suggest that variants in the newly discovered exons studied do not contribute to Rett syndrome, furthermore if some of these variants are related to a phenotype, this must be different from Rett. Interestingly however, the variants in the novel exons are twice as frequent as those found in flanking sequences (50 vs 24 for approximately 1.3 Mb sequenced for each class of sequences). The significance of this result remains to be elucidated; one hypothesis is that novel exons accumulate variants faster than the rest of the genome (positive selection?) that could underscore the functional importance of these sequences.

**P01.115****Rett syndrome cases with more than one causative mutation in the MECP2 gene**

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Rett syndrome is an X-linked dominant disease affecting females. It is a progressive neurological disorder characterized by normal birth and psychomotor development for the first six to 18 months of life followed by severe regression in motor and language ability. In 80% of cases mutations in the Methyl-CpG-binding protein 2 (MECP2) gene are identified, usually de novo point mutations, however up to 16% are caused by deletions of one or more exons of the gene. Almost all are single cases in a family resulting from de novo mutations or the inheritance of a mutation from an unaffected parent with somatic or germ line mosaicism.

Point mutation testing and gene dosage analysis of a cohort of 455 cases referred to our laboratory for MECP2 gene analysis identified mutations in 118 cases. However there were four females who each had two different de novo causative mutations, presumed to be on the same chromosome because compound heterozygosity for two causative mutations is likely to be lethal. Two of these cases had a point mutation and a small intraexonic deletion, a third had a whole exon deletion and a separate small intraexonic deletion and a fourth had a small intraexonic deletion and a large duplication. These findings highlight the necessity to perform both point mutation and exon dosage analysis in such cases, particularly because of the possibility of undetected parental mosaicism and the implications for prenatal diagnosis in future pregnancies. These cases also suggest that the MECP2 gene may be particularly prone to mutation.

**P01.116****FRAXA Molecular Genetic Diagnosis of the oocyte' donor population during 2007 for Assisted Reproduction Treatments.**

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The Instituto Valenciano de Infertilidad (IVI) has implemented for the oocyte donation program in our clinics, the routine study for molecular analysis of X-FRAGILE Syndrome (FRAXA). FRAXA is one of the greatest genetic prevalence illnesses in general population. In this communication, we present our analysis, using the protocol recently developed by Abbott Molecular, called Fragile X PCR Test,. We studied 2278 women from different cities in Spain. All these women as susceptible oocyte'donors for processing of assisted reproductive treatments (TRA).

FRAXA is the most common cause associated to mental family inherited and represents among 15 to 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 it's methylation and repression of expression. According repetitions number of the CGG tri-nucleotide, this region is considered normal (<50 CGGn), premuted (55-200 CGGn) or full mutated (>200 CGGn).

In this poster we present the results of this extense study. In conclusion, we found 21 (0,92 % ) premutation carriers; 49 (2,15%) "intermediate" carriers and 1/33 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. On the other hand, 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.

#### P01.117

#### Genotype-phenotype correlation in Rubinstein-Taybi syndrome

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The Rubinstein-Taybi Syndrome is an autosomal dominant disease. It is characterised by typical facial dysmorphism, abnormalities of the extremities, growth retardation and psychomotor development delay. Mutations have been identified in two genes, *CREBBP* and *EP300*. We report a series comprising 93 patients with *CREBBP* mutations, and 2 patients with *EP300* mutations. Point mutations were searched for by DHPLC followed by sequencing, and microrearrangements were identified by array-CGH and/or Quantitative Multiplex Fluorescent PCR (QMF-PCR). Among the *CREBBP* mutations, 24% were nonsense, 24% led to a translation reading frame shift, 26% were microrearrangements (24% deletions, 2% duplications), 18% affected a splice site, and 8% were missense. It is noteworthy that no mutation was ever found in exons 7, 9, 11, 18, 22, 23 and 29. Whatever the mutation type and localisation along the gene, the dysmorphic and osseous phenotypes were comparable to the typical RTS phenotype. However, patients carrying a nonsense mutation presented associated anomalies such as cardiopathy (33% vs 26%) or neurological anomalies (hypotonia in 81% vs 60%, hyperreflexia in 37% vs 29%) more frequently than patients with any other type of mutation. Conversely, patients carrying missense mutations presented with less severe phenotypes, since none of them presented any associated malformation or anomaly.

Concerning the 2 patients with an *EP300* mutation, our data were in accordance with those of the literature, since they presented with a typical dysmorphic phenotype with a less severe osseous phenotype. Only one of the two patients had broad thumbs and halluces, without any associated osseous anomaly.

#### P01.118

#### Chromosomal imbalances in Rubinstein-Taybi patients negative to CREBBP mutational test

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Rubinstein-Taybi syndrome (RSTS, OMIM #180849) is a rare autosomal dominant congenital disorder characterized by postnatal growth retardation and psychomotor developmental delay, skeletal anomalies and specific facial dysmorphisms. RSTS is associated with chromosomal microdeletion or point mutations of *CREBBP* gene in 16p13.3 and

mutations of *EP300* gene in 22q13, observed in 56% and 3% of the tested patients respectively.

Here we report the identification by a-CGH of duplications/deletions in 6 of 25 RSTS patients found negative to point mutations of *CREBBP* and to chromosomal rearrangements affecting *CREBBP* and *EP300* regions. The imbalances are: i) a de novo 9Mb deletion in 2q24.3q31.1 involving the HOXD genes, ii) a 5,5 Mb duplication in 2q34q35 inherited by the healthy father, probably representing a private CNV, iii) a 500Kb duplication in 17q11.2 upstream the *NF1* gene in a region delimited by *NF1* REP-P1/P2 iv) a 1,2Mb deletion in 18q21.33q22.1 harbouring one gene, v) a 4,3Mb deletion in 2q22.3q23.1 involving five genes among which *ZFHXB1*, the gene mutated in Mowat-Wilson, vi) a 466Kb deletion in 7p21.1 containing *TWIST1*, a proposed candidate for RSTS. The parental origin, gene content and genomic characterization of the last four imbalances is in progress. Although the pathogenetic role is yet unproven in a few cases, this study shows a high fraction of chromosomal rearrangements in regions other than those of *CREBBP/EP300* genes. In addition a-CGH is confirmed to be a suitable approach for diagnostic purposes and to highlight novel positional RSTS candidate genes. Supported by A.S.M. (Associazione Italiana Studio Malformazioni).

#### P01.119

#### Two cases of Rubinstein Taybi Syndrome caused by two new mutations that create premature stop codons and truncated CREBBP protein

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<sup>1</sup>Genetica Medica Dipartimento di Scienze della Riproduzione e Sviluppo, Trieste, Italy, <sup>2</sup>Genetica Medica. Università degli Studi di Milano, Ospedale San Paolo, Milano, Italy, <sup>3</sup>Genetica Medica, IRCCS Burlo Garofolo, Trieste, Italy. Rubinstein-Taybi syndrome (RSTS; MIM 180849) is a congenital disorder characterized by growth retardation and psychomotor delay, broad thumbs and halluces and a wide range of dysmorphic features. RSTS was shown to be associated with microdeletions and point mutations in the gene encoding the CREB-binding protein, located in 16p13.3. The first case was born from a twin pregnancy. While the sister was normal during pregnancy and postnatal period, the patient had IUGR and typical dysmorphic features since the first weeks of life. Cardiac valvular anomalies were found as well as a bilateral conductive hypoacusia. RSTS was then suspected and was confirmed by molecular analysis. A new mutation was found: c.5838\_5857dup20, with the formation of a premature stop codon (p.Pro1953HisfsX30) resulting in a truncated protein of 1982 aminoacids. Second case was an Haitian girl. Pregnancy was terminated at 32 weeks for preeclampsia and IUGR. She was hospitalized because of respiratory distress, feeding difficulties and severe growth retardation. A recurrent infection condition secondary to gastroesophageal reflux was suspected. However, encephalic NMR demonstrated corpus callosum agenesis; oculistic examination revealed severe strabismus and nasolacrimal duct obstruction and bone age was marked delayed. Furthermore, physical examination demonstrated typical RSTS dysmorphisms. Molecular analysis of the *CREBBP* gene revealed a 7 nucleotide deletion: c.3608\_3609+5del, eliminating the donor splicing site of exon 18 with the formation of a premature stop codon. This mutation produces a truncated protein (1204 aminoacids). The identification of these two new alleles may increase the knowledge of *CREBBP* function and the prediction of genotype-phenotype correlation.

#### P01.120

#### A predominantly sensorial case of CHARGE: towards a refinement of CHD7 disease spectrum

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Introduction: We present here a novel *CHD7* mutation in a mildly affected boy. The implications for further delineation of *CHD7* disease spectrum are underlined.

Clinical case: BM was born from unrelated Caucasian parents, with microphallus and bilateral cryptorchidism. Hypogonadotropic hypogonadism was diagnosed in neonatal period. Ophthalmologic examination revealed relative microptalmia with right chorioretinal coloboma and left retroretinal cysts. Psychomotor development and cardiac ul-

trasound were normal. A small isolated notch was noted on the right pinna.

At age 4, right sensorineural deafness was found and cranial imaging revealed bilateral middle and internal ear abnormalities (Mondini defect, bilateral semicircular canal agenesis, large sacculus, ossicular chain ankylosis), and hypoplastic olfactory tractus, without median line defect.

At age 10, anosmia was diagnosed. Growth was conserved, except for delay due to absence of puberty, and he performed well at school.

At age 13, screening of *CHD7* by heteroduplex and sequence analysis detected a c.6290A>G (p.Asp2097Gly) heterozygous mutation. Parents have not yet been tested but different models predict a probable pathogenic effect of this substitution.

**Conclusions:** This presentation suggests that CHARGE syndrome (MIM 214800) has to be evoked in children with apparently isolated sensorial deficiencies, and certainly in those with unilateral deafness and inner ear radiological abnormalities, in agreement with recent clinical criteria (Verloes). *CHD7* mutation screening would be helpful for better characterization of these "predominantly sensorial" forms, and if confirmed, could allow a more specific work-up and management, with regards to the expanding knowledge on *CHD7* disease spectrum (neurological, mainly rhombencephalic, endocrinological and immunological deficiencies).

#### P01.121

#### Clinical and molecular characterisation of a cohort of Portuguese CHARGE patients

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<sup>1</sup>Serviço de Genética do Hospital de Santa Maria, Lisboa, Portugal, <sup>2</sup>Duncan Guthrie Institute, Yorkhill, Glasgow, United Kingdom, <sup>3</sup>Serviço de Pediatria do Hospital Fernando da Fonseca, Lisboa, Portugal, <sup>4</sup>GenoMed, Lisboa, Portugal. CHARGE is an acronym standing for Coloboma, Heart malformation, choanal Atresia, Retardation of growth and/or development, Genital anomalies and Ear anomalies. Since the original description, other clinical features have been added to the CHARGE spectrum of anomalies, namely rhombencephalic dysfunction, hypoplasia of the semi-circular canals and arhinencephaly. The diagnostic criteria now stress the importance of the 3C triad (Coloboma, Choanal atresia, abnormal semicircular Canals). Additionally, patients are classified as typical (3 major or 2 major and 2 minor criteria), partial or incomplete (2 major and 1 minor criteria), or atypical (2 major or 1 major and 2 minor criteria). CHARGE was considered an association until recent years, when *CHD7* mutations were identified in approximately 2/3 of patients.

We reviewed the patients with a clinical diagnosis of CHARGE syndrome who visited our Genetics Clinic. 10 patients met the most recent diagnostic criteria. 5 cases could be classified as typical CHARGE and 5 cases were atypical. However, 3/5 of the latter were not checked for hypoplasia of the semicircular canals, which could well alter their status.

*CHD7* mutation detection studies were undertaken in all these patients, except for one of the typical CHARGE cases who died in the neonatal period. Highly likely causative mutations were identified in 7 patients overall, 3/4 typical and 4/5 atypical cases. In the remaining atypical CHARGE patient a sequence change of unknown clinical significance was found. This variant was also present in the patient's mother, who has subtle facial asymmetry and anisocoria, complicating result interpretation.

#### P01.122

#### A prenatal diagnosis of CHARGE syndrome

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We report on a female foetus in whom a CHARGE syndrome was diagnosed at 31 weeks of gestation. A Fallot tetralogy and a cleft lip and palate were observed at a fetal ultrasound examination performed

at ~23 weeks of pregnancy. Amniocentesis, performed in another hospital, revealed a normal female karyotype. A control of the ultrasound examination at 29 weeks' gestation showed, in addition, the presence of dysplastic cup-shaped ears, especially at the right side, evoking the diagnosis of CHARGE syndrome. Fetal brain magnetic resonance was suggestive for the presence of hypoplastic semi-circular canals, a finding that was confirmed by a fetal CT scan of the inner ear.

The parents were young, healthy and non-consanguineous. Family history was unremarkable, except for the presence of miscarriages. The parents were informed about CHARGE syndrome and decided to continue the pregnancy. The child, born at 37 5/7 weeks, had a left-sided cleft lip and palate, a heart murmur 3/6, a facial asymmetry (with right eye being smaller than the left eye) and typical CHARGE ears. She had major cardio-respiratory problems and died soon after birth. The parents refused post-mortem examination, but a cerebral brain MRI could be performed post-mortem and showed a coloboma of the left retina and absent olfactory bulbs. Blood analyses for *CHD7* mutational screening are ongoing.

This case illustrates the use of both fetal brain MRI and CT scan of the inner ear in establishing a prenatal diagnosis of CHARGE syndrome.

#### P01.123

#### A novel SOX9 mutation in a case with camptomelic dysplasia

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Camptomelic dysplasia (CD, MIM 114290)) is a rare, often lethal, dominantly inherited, congenital osteochondrodysplasia, associated with male-to-female autosomal sex reversal in two-thirds of the affected karyotypic males. Prominent features are bowing and angulations of long bones, Robin sequence, pelvis, chest and rib abnormalities. De novo mutations of the *SOX9* gene, a tissue-specific transcription factor gene involved both in skeletogenesis and male sexual differentiation, are known to be responsible for both CD and XY sex reversal. Here we present a 4-month-old infant of young non-consanguineous parents out of the Turkish minority group in Bulgaria. Femoral malformations and macrocephaly were detected prenatally. The newborn was delivered at term and presented with an extremely short birth length, below -7 SD, dysmorphic facial features, median cleft palate, remarkably bowed and short limbs, narrow asymmetric thorax, club feet, short deformed toes, and radiographic features characteristic for camptomelic dysplasia. Male pseudohermaphroditism with female external genitalia, enlarged clitoris, and male karyotype was found. DNA analyses revealed a novel de novo mutation of the *SOX9* gene, Q401X. A similar nonsense mutation, Y400X, has been described previously (Hum Mol Genet 6:91, 1997). In both cases, a truncated *SOX9* protein results that completely lacks the C-terminal transactivation domain (residues 402-509). The Q401X mutant *SOX9* protein will still be able to bind to DNA, as it still retains the DNA-binding domain, but will be unable to activate transcription of the genes that are regulated by this transcription factor.

#### P01.124

#### Opsismodysplasia with renal agenesis: A case report

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Opsismodysplasia is a rare chondrodysplasia clinically characterised by micromelia, respiratory distress, and major delay in skeletal ossification.

We present a novel case of opsismodysplasia in a foetus after termination of pregnancy at 30 weeks of gestation. Prenatal was diagnosed during ultrasound in our clinic with shortness of the long bones and short extremities, depressed nasal bridge, narrow thorax. The parent's ethnic origin was Roman, no information about the family consanguinity. After termination of pregnancy the female newborn lived only 30 minutes. She was at birth: the weight 840g, the length 29 cm, PC 25 cm

and PT 19 cm. Necropsy confirmed dysmorphic features, coarse face, and showed bilateral pulmonary hypoplasia, bilateral renal agenesis (not previously reported in opismodysplasia) rhizomelic micromelia (handwriting appearance of hands fingers).

She presented: high forehead, horizontals and short palpebral fissures, posterior rotated and low placed proeminent ears, small anteverted nose with long philtrum, depressed bridge of nose, micrognathia, thin upper lip, muscle hypotonia , short feet and hands, abnormalities of fingers, dorsolumbar scoliosis. Radiographic findings were hypoplastic vertebral bodies, marked shortness of the long bones of the hands and feet with concave metaphyses.

Karyotype from the peripheral blood lymphocytes and G banding were performed according to standard protocols. The karyotype was 46,XX. In our knowledge were reported only ten cases with termination of pregnancies after the antenatal diagnoses and this is the first case of opismodysplasia associated with bilateral renal agenesis .

#### P01.125

#### New ocular findings among two sisters with Yunis-Varón syndrome

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**Introduction.** The Yunis-Varón syndrome (YVS) represents a rare autosomal recessive syndrome characterized by cleidocranial dysplasia, absence of thumbs and halluces, distal aphalangia, ectodermal anomalies, and poor outcome. This report is among two sisters with YVS, which adds ocular findings to the known features of this syndrome.

**Clinical reports.** The first patient, a 11-month-old female infant was the third child of a healthy consanguineous parents of Mexican descent. She has pre- and post-natal growth retardation, wide sutures and fontanelles, sparse hair, telecanthus, short upper lip, high arched palate, abnormal ears, micrognathia, loose skin in neck, sloping shoulders, hypoplastic thumbs, distal aphalangia, nail hypoplasia, aplasia of great toes, and short pointed toes. Besides, skull dystostosis, hypoplastic clavicles, hypoplasia of proximal phalanges, agenesis of distal phalanges, congenital cardiopathy, central nervous system anomalies, hearing loss and visual impairment were also observed. Ophthalmologic evaluation and fluorescein angiography showed papillo-macular atrophic chorioretinopathy with "salt-and-pepper" appearance. Results of TORCH titers, metabolic screening test, karyotype, and muscle biopsy were normal or negative. The second patient was a female newborn with physical, ophthalmologic, and radiological findings similar to her sister. Absence of thumbs and first toes were observed on ultrasound examination at 22 weeks.

**Conclusions.** This appears to be the first cases of YVS associated with chorioretinopathy that could not be attributed to environmental causes. Other anomalies such as sclerocornea, cataracts, corneal opacity and microphthalmos make mandatory the ophthalmologic evaluation for patients with YVS.

#### P01.126

#### COL2A1 mutations and related phenotypes in Italian patients with presumptive type II collagenopathy

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COL2A1 mutations are associated with a wide spectrum of phenotypic manifestations. At present little is known about the distribution of COL2A1 defects and related phenotypes in Italian patients. We investigated a series of 13 unrelated Italian probands with a presumptive diagnosis of type II collagenopathy. The 54 exons of the COL2A1 gene

were screened by dHPLC, followed by sequencing of amplicons with an abnormal profile. Overall, eight distinct mutations were detected. Five of these had been previously described in the literature, and three have never been reported to our knowledge. Previously described genotype-phenotype correlations for the already reported mutations were confirmed in this series.

Among the newly reported mutations, the IVS7+1G>A variant was found in a female proband with typical type 1 Stickler syndrome with a positive family history and wide intrafamilial phenotypic variability. A nonsense mutation, K1447X, in the C-terminal region of the protein was found in a family, mother and child, studied for a suspicion of Kniest Dysplasia. Finally, a G891C missense variant, affecting the triple helical domain of the protein, was associated with a clinical picture of spondyloepimetaphyseal dysplasia, Strudwick type. Further cases will need to be examined in order to obtain a better definition of genotype-phenotype correlations for COL2A1 mutations in the Italian population.

#### P01.127

#### Genetic diagnosis of the craniosynostosis in Spain

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The craniosynostosis consist in an anomalous fusion of cranial bones that originates problems in the normal growth of the cranium and involves dramatic alterations in the shape of the head and the face, and in some cases severe mental delay. The craniosynostosis is classified under several syndromes: Apert, Crouzon, Pfeiffer, Beare-Stevenson, Saethre-Chotzen, Muenke and Jackson-Weiss. Each one has a particular pattern of inheritance and a few clinical specific characteristics, but in some cases they are difficult to distinguish by the external aspect, specially when prenatal diagnosis is done by means of the ultrasound scans. The genetic diagnosis allows to detect the craniosynostosis in the first stages of the pregnancy and to confirm the clinical observations.

The most important genes associated to these disorders are FGFR1, FGFR 2, FGFR3 and TWIST. The genetic study of the craniosynostosis is complex, and may includes the four genes involved in the pathology. In this communication we show the results obtained in the study of these syndromes in Spain. We analysed 38 genes from 19 people, including affected people and their families. We found 5 mutations that have been reported as the genetic basis of the disease. We also done a prenatal genetic diagnosis that confirms a Pfeiffer syndrome, previously diagnosed by ultrasound scan. Our results suggest that most of mutation associated to craniosynostosis are located in a few regions of these genes, in opposition to what happens with other genetic diseases, in which the mutations could be found along the entire gene.

#### P01.128

#### Czech dysplasia: clinical and molecular delineation of a novel COL2A1 disorder

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Czech dysplasia (OMIM 609162) is a novel type II collagen disorder which is phenotypically distinct from other COL2A1-related diseases. Czech dysplasia is characterized by early-onset progressive pseudorheumatoid arthritis, short third and fourth metatarsals, mild platyspondyly, normal height, and the absence of ophthalmological problems or cleft palate. The disorder is caused by a specific missense mutation (R275C, c.823C>T) in the triple helical domain of the COL2A1 gene. We report a large German family consisting of 11 patients who not only suffered from the typical features mentioned above, but who also had sensorineural hearing loss, a problem that has hitherto not been considered as a major feature of Czech dysplasia. Mutation analysis revealed the COL2A1 c.823C>T (R275C) mutation in all patients. This finding provides further evidence that Czech dysplasia is caused exclusively by the R275C mutation, which is a unique situation among the COL2A1 disorders, and indicates that this amino acid exchange leads to specific structural changes of type II collagen. The clinical and radiological data of this family and previously reported patients with

the R275C mutation demonstrate a remarkably uniform manifestation of the pathological features and add hearing loss to the list of major problems of Czech dysplasia.

#### P01.129

#### Clinical and molecular characterization of Diastrophic Dysplasia in the Portuguese population

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Diastrophic dysplasia (DTD; MIM #222600) is an autosomal recessive chondrodysplasia, characterized by limb shortening, hitchhiker thumbs, spinal deformities and contractures. Diagnosis is confirmed by molecular testing of the sulphate transporter *SLC26A2* gene. *SLC26A2*-related dysplasias encompasses a range of disease: from lethal achondrogenesis type 1B and atelosteogenesis type 2 (AO2) to classical DTD and mild recessive multiple epiphyseal dysplasia (rMED). Genotype-phenotype correlations have been described. This study aimed at characterizing clinically, radiologically and molecularly, 14 patients and 3 foetuses affected by *SLC26A2*-related dysplasias and to evaluate genotype-phenotype correlation. The main Portuguese Departments of Genetics and Orthopaedics were contacted in order to recruit patients from all the country.

Phenotypically, 8 patients were classified as classical DTD, 3 patients as rMED, and 3 patients had an intermediate phenotype (mild DTD). Foetuses had a homogenous presentation of severe DTD/AO2. Molecular analysis showed that the R279W mutation is present in all living patients, in homozygosity in rMED and in compound heterozygosity with the known severe allele R178X in classical DTD.

This report shows the clinical and molecular spectrum of *SLC26A2*-related skeletal dysplasias in the Portuguese population. R279W mutation in homozygosity causes rMED and the association of this mild allele with a null mutation causes classical DTD. The "Finnish mutation", was not found and is probably very rare in the Portuguese population. The data of this series indicate that screening for common *SLC26A2* mutations allows to confirm the diagnosis in the majority of Portuguese patients or at least to identify one pathogenic allele.

#### P01.130

#### Compound heterozygosity for GDF5 in Du Pan type chondrodysplasia

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Du Pan type Chondrodysplasia (DPC) represents the milder end of the Growth Differentiation Factor 5 (GDF5) morphopathies which, according to an increasing gradient of clinical severity, include also the Hunter-Thompson type and the Grebe type acromesomelic chondrodysplasias. DPC is characterised by mild short stature, complex brachydactyly, and fibular aplasia. Affected individuals are homozygotes for loss-of-function mutations in the highly conserved mature domain of GDF5 that result in an impaired GDF5 signaling through the Bone Morphogenic Protein Receptor 1B (BMPR1B). We investigated a 20 month-old child with complex brachydactyly and mild proximal fibular hypoplasia, consistent with DPC, in the absence of short stature and other long bones' and joints' anomalies. Mutational analysis disclosed compound heterozygosity for two novel GDF5 mutations, the P436T mutation in the mature domain, that most likely results in reduced binding to BMPR1B and the R378Q mutation at the end of the prodomain. The mutation R378Q is located within the recognition motif at the processing site

of GDF5 where the precursor protein is cleaved and probably results in at least a partial loss of protein function. Metacarpophalangeal profile (MCPP) analysis on radiographs of the parents disclosed unremarkable results for the P436T mutation, transmitted from the mother, while the father's R378Q mutation was associated with a MCPP showing overlap with BDA1, including brachymesophalangy of all digits and mild shortening of the entire first digit. This observation connects DPC with the BDA1 phenotype and further expands the clinical variability of GDF5 associated phenotypes.

#### P01.131

#### Early recognition of Ellis van Creveld syndrome: report of a new case

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Ellis van Creveld is a rare chondro-ectodermal dysplasia characterized by short ribs, polydactyly, growth retardation and ectodermal defects. Significant findings in the general survey of our male case were narrow thorax, postaxial polydactyly of the hands, bone deformities with extremities markedly shortened, dysplastic fingernails, neonatal teeth, respiratory distress due to narrow chest.

Aspects of the ribs and dystrophic nails are the major criteria in establishing the diagnosis. It may be differentiated from other chondrodystrophies or syndromes associated with polydactyly.

Early recognition is important in order to identify the real diagnosis, to know evolution and prognosis and to offer proper genetic counselling. Prognosis of our case is linked to the respiratory difficulties.

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#### P01.132

#### New molecular findings in Hypochondroplasia: clinical, radiological and molecular review of a cohort of 15 Portuguese patients

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Short stature in childhood is a frequent clinical presentation, being 1% of these cases due to bone dysplasias. Achondroplasia (Ach;MIM#100800)/ Hypochondroplasia (Hch;MIM#146000) are the two most common autosomal dominant skeletal dysplasias. FGFR3 gene is the only currently known gene to be implicated.

In Hch, p.N540K mutation in tyrosine-kinase domain 1 accounts for up to 65% of cases.

These two conditions have overlapping phenotypes characterized by disproportionate short stature with rhizomelic limb shortening, Ach being more severe.

We reviewed 13 probands and their first relatives (16 patients) followed at our Genetics Clinic with diagnosis of Hch. Clinical and radiological findings were assessed independently by two clinical geneticists. Differential diagnosis were considered and systematically excluded whenever indicated.

Molecular study of FGFR3 gene was performed in all cases.

Seven mutations were identified in 16 patients: three N540K, one rare mutation R200C (described once) and a new mutation p.E360K in a pair of sibs and their mother.

When there is a high (clinical/radiological) suspicion of hypochondroplasia, and after negative mutation scanning, full sequencing FGFR3 coding region is indicated.

This allows a higher detection rate uncovering rare/new mutations. In our series one new mutation and another rare one were identified by this method.

Sequencing of FGFR3 coding region was negative in 7 patients. These patients also have clinical and radiological features within the spectrum of Hch. In these patients hypochondroplasia is most probably not FGFR3 related, raising attention to the role of other unknown (at the moment) genes may play in this condition.

**P01.133****White matter abnormalities in siblings with Goldberg Shprintzen syndrome**

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Goldberg Shprintzen syndrome (OMIM 609460, GOLDBERG-SHPRINTZEN MEGACOLON SYNDROME - GOSHS) is rare syndrome of congenital malformations with autosomal recessive pattern of inheritance. The most frequent manifestations are: mental retardation, microcephaly with accompanying dysmorphic features, and Hirschsprung disease. The syndrome is a result of mutations in KIAA1279 gene, which function is still poorly understood. It is possible that incorrect function of the gene product is a cause of abnormal migration of neurons, thus in addition to Hirschsprung disease, a brain developmental defects e.g. polymicrogyria, pachygryria or agenesis of corpus callosum is observed. There is just few reports in the literature describing GOSHS, including one reporting an abnormal intensity of white matter signals. Here we report two brothers with GOSHS symptoms and mutation fund in KIAA1279 gene. The MRI revealed in one patient evidence of cranio-facial dysmorphology, asymmetry and dilatation of the ventricular system, and partial agenesis of corpus callosum. Additionally, in both patients discreet stranded regions of high intensity signals along the Roland's sulci, in the both internal capsules and lateral parts of the thalamus were found. Also, in both patients abnormal values of evoked potentials were observed. The clinical symptoms were variable in GOSHS, similarly like in most such syndromes. In one patient the features of Hirschsprung's disease did not occur but in the other one they were of mild intensity. Changes in the MRI pattern of the brain not seen in this syndrome until now and systematic description of clinical features are valuable additions to clinical characterization of GOSHS.

**P01.134****Two new cases of Hajdu-Cheney Syndrome and further syndrome delineation**

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Hajdu-Cheney syndrome (HCS) (102500#OMIM) is a rare disorder characterised clinically by small stature and distinctive face. Diagnostic radiographic features consist of acro-osteolysis and unique dysplastic skull changes. We report on two new HCS cases a 9 and 16-year-old boys full filling inclusion criteria by Brennan and Pauli (2001). A detailed phenotype description of each boy is given and evaluated together with 23 other published case reports with the aim to delineate the spectrum of clinical and anthropological features. A catalogue of nearly 900 well-defined traits according to Stengel-Rutkowski *et al* (1996) with own modification was used. Sixty dysmorphological, clinical and radiographic features were put into the quantitative phenotype definition of HCS syndrome. Additionally, a variety of clinical (hearing loss, vocal scale limited to lower tones, delayed fontanel closure, delayed dental eruption, open bite, depressed sternum) and radiological findings (Wormian bones, acro-osteolysis, generalized osteoporosis) were taken into account. As molecular basis of this entity remains unknown, we believe that quantitative phenotype definition of HCS can be helpful for early diagnosis, before acro-osteolysis, the decisive diagnostic sign, develops.

**P01.135****Familial Hanhart Syndrome in a Newfoundland Kindred**

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Hanhart syndrome, also known as oligodactyly-hypoglossia, adactyly-aglossia or oromandibulo limb hypoplasia syndrome because of the cardinal signs of limb and tongue anomalies associated with abnormal mandibular development, is a rare genetic condition that usually happens sporadically and is assumed to be the consequences of either a vascular disruption event or a new dominant mutation. A Newfound-

land kindred spanning 4 generations is believed to have this condition where affected members manifest ulnar ray defects, oligodactyly and ankyloglossia with highly variable expressivity amongst the affected. Development, intelligence, stature and overall health are normal for these individuals. To my knowledge, this is the first report of familial cases of this genetic condition. Its presence confirms autosomal dominant inheritance as one underlying etiology.

**P01.136****Holt-Oram syndrome - case report**

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Holt-Oram syndrome (HOS) is an autosomal dominant condition with variable expressivity characterized by the association of congenital heart defects and preaxial radial ray upper limb defects. We present a new case in order to illustrate this rare entity and to discuss the variable expression and the management. Our proband is a 13,5 old male, the only child of an unrelated couple. Father presents absence of the left thumb, short left limb, cardiac failure. No fetal ultrasound scan was performed. The proband was born naturally at 37 weeks gestation (Wt-2450g, Ht-46,5cm, HC-36 cm, Apgar score 7). Postnatal development was relatively normal. Physical examination (13y old) revealed: Wt - 2,52 SD, short limbs with absence of forearms, bilateral absence of thumbs, absence of left forefinger. Radiological examination showed bilateral absence of radius and ulna. Echocardiography: ASD ostium secundum; ECG: first-degree atrioventricular block. We have established the diagnosis of HOS based on the characteristic association of congenital heart defects and upper limbs defects. Differential diagnosis was done with other heart-hand syndrome. The plan for the management and the genetic counseling will be presented. In conclusion, we present a case of HOS in order to illustrate this rare genetic disorder but also to discuss the variable expression, the management and the genetic counseling.

**P01.137****Spinal abnormalities, Klippel-Feil syndrome and the Mayer-Rokitansky-Küster-Hauser syndrome: four case reports**

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The Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome is characterized by congenital aplasia of the uterus and the upper part (2/3) of the vagina and is regarded as an inhibitory malformation of the Müllerian ducts. MRKH may be isolated but it is more frequently associated with renal, vertebral, and, to a lesser extent, auditory and cardiac defects (MURCS association). The molecular basis for the MRKH syndrome is currently unknown.

We describe four female patients in whom diagnosis of MRKH syndrome was confirmed. All complained of primary amenorrhea associated with cyclic pelvic pain and presented normal sexual development, normal levels of FSH, LH and 17beta estradiol, 46,XX karyotype and congenital absence of the uterus and upper vagina.

The first patient presented short neck, low-set posterior hairline, limited neck motion and fusion of cervical vertebrae associated with unilateral elevated scapula. The second patient presented low-set posterior hairline, short neck with very limited neck motion and fusion of cervical vertebrae associated with unilateral elevated scapula, cyphoscoliosis, asymmetrical breast development, dental abnormalities and flat feet. A familial history with a Sister complaining of secondary amenorrhea with low-set posterior hairline, minor scoliosis and flatfeet, was recorded. The described skeletal deformity in these 2 females, were consistent with Klippel-Feil syndrome.

The third patient presented low-set posterior hairline, short neck, shield-like chest and scoliosis. She presented also dental deformities, *cafe-au-lait* spots and many nevus spilus and vasculitis. An history of two azoospermic maternal uncles was recorded. The forth patient presented a minor scoliosis and asymmetrical hips.

**P01.138****Clinical and molecular diagnosis of ADULT syndrome in a pregnant woman**

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A 29 year old woman affected by ectodermal dysplasia came to our attention during her 1st pregnancy, asking about the risk of recurrence in her children and the possibility of diagnostic prenatal testing.

No relatives were reported to be affected and the patient's parents were not consanguineous. Ectodermal dysplasia was suspected in childhood because of hypodontia, corneal dystrophy, sparse hair, thin skin and dystrophic nails, but a specific diagnosis had never been formulated.

Upon clinical examination we noticed, besides classical signs, mild dysmorphic features: deep-set eyes with upward-slanted and short palpebral fissures, high forehead, low nasal bridge. Moreover, we noticed bifid uvula, freckling and absence of nipples, which led us to hypothesize involvement of the p63 gene. In fact, molecular analysis of p63 identified the presence of a Gly134Val mutation, previously described in a patient with ADULT syndrome. p63 mutations can give rise to a spectrum of partially overlapping phenotypes, from ADULT syndrome to limb-mammary and EEC syndrome, all transmitted with an autosomal dominant pattern.

Absence of the mutation in the parents proved its de novo origin. Our patient underwent CVS for fetal molecular analysis and, fortunately, the mutation was not transmitted to the fetus.

Due to limited time availability, it is seldom possible to successfully resolve a molecular diagnosis during pregnancy without preconceptional analysis. Thanks to careful clinical examination, the availability of an efficient laboratory and prompt familial involvement, our proband received the result of prenatal testing in less than one month from the 1st counselling session.

**P01.139****Variable expression in a familial case of Pfeiffer Syndrome with FGFR1 P252R mutation**

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A 4 year and six month old boy was diagnosed with Pfeiffer syndrome on the basis of bilateral coronal craniostenosis, skin syndactyly of toes, brachydactyly, broad thumbs and big toes and characteristic facial dysmorphisms.

Close examination of the patient's family revealed flattening, bilateral soft tissue hypertrophy, foreshortening and medial deviation of the great toes as well as mild bilateral proptosis in the mother and in the older brother. The brother also had partial skin syndactyly of toes, broad thumbs and camptodactyly of the left fifth finger. Neither the mother, nor the brother showed any signs of craniostenosis.

Molecular analysis of the FGFR1 gene revealed the P252R mutation in all three family members.

Several articles have documented the high clinical variability and genetic heterogeneity in individuals affected by Pfeiffer syndrome.

As far as we know this is the first case of an affected family with the common FGFR1 P252R mutation in which just the proband has the full-blown syndrome, whereas the other two affected persons show characteristic feet anomalies without skull involvement, underlining the broad intrafamilial variability of expression.

**P01.140****Molecular analysis of a Case of Pfeiffer Syndrome**

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Pfeiffer syndrome is a rare autosomal dominantly inherited osteochondrodysplasia with craniostenosis conditions that is genetically heterogeneous that can be caused by mutations in the fibroblast growth factor receptor 2 (FGFR2) gene. The FGFR2 gene provides instructions for making a protein called fibroblast growth factor receptor 2. More than 20 FGFR2 mutations that cause Pfeiffer syndrome have

been identified. Several of these mutations change the number of cysteine amino acids in a critical region of the FGFR2 protein known as the IgIII domain.

Sporadic cases of Pfeiffer syndrome have previously been associated with advanced paternal age.

The patient in our study was a 2 years old male baby. His parents were healthy both under 25 years and they were not consanguineous. Labor began spontaneously and the infant was delivery vaginally at term with a birth weight of 2,975 g with the Apgar score of 8-9. The propositus had a cloverleaf skull, severe exorbitism, choanal atresia, low-set and posteriorly-rotated ears, broad and medially-deviated halluces and partial cutaneous syndactyly of the second and third toes. The ocular globes and eyelids were intact with shallow orbits that would have prevented the replacement of the eye. The ocular anterior structures were preserved, without iris or lens abnormalities. The baby developed respiratory distress.

Molecular analysis of the patient revealed a heterozygous C342R mutation in exon 10 and a missense T→C in mRNA further studies on such sporadic cases recommended enhancing the molecular information.

**P01.141****Combination of Saethre-Chotzen and Greig syndromes in one family: the phenotype of a proband**

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We present a female child (5-year-old), from a family with the features of cephalopolysyndactyly (Greig syndrome) in her mother (30-year-old), and acrocephalosyndactyly (Saethre-Chotzen syndrome) in her father (28-year-old) and paternal grandfather (61-year-old). Both syndromes are autosomal dominant, minimal diagnostic criteria for the first are syndactyly, polydactyly and cranial anomalies, for the second - cranial synostosis with skull asymmetry, 2-3 toes syndactyly. The mother's phenotype consisted of cranial anomalies (protrudent frontal tubers, hypertelorism, wide bridge of the nose), anomalies of extremities (postaxial polydactyly of the right hand; moderately wide nail bones of the first fingers of both hands; preaxial polydactyly and full 1-2-3 toes syndactyly of the right foot; preaxial polydactyly with total toes hypoplasia and full 1-2-3 toes syndactyly of the left foot). Clinical features of both the father and the grandfather included macrocephalia, facial asymmetry, nasal septum deviation, partial 2-3 toes syndactyly. The child showed a combination of cranial anomalies (macrocephalia, facial asymmetry, nasal septum deviation, antimongoloid slant, wide bridge of the nose), epicanthus of both eyes, low-set ears, anomalies of extremities (postaxial polydactyly, preaxial polydactyly with full syndactyly and common nail plate of both hands; postaxial polydactyly, partial 2-3 and 4-5 toes syndactyly, full 3-4 toes syndactyly of the right foot; postaxial polydactyly and full 2-3-4 toes syndactyly of the left foot). Thus, the proband has inherited the signs of both syndromes, this means realization of their 50% of probabilities. The fact significantly increases the risk for further generations, which should be taken into account during genetic counseling.

**P01.142****Short rib-polydactyly syndrome on three consecutive pregnancies**

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Short rib polydactyly syndromes are lethal skeletal dysplasias with an autosomal recessive inheritance pattern that can be distinguished on radiological and histological grounds. We report on three consecutive pregnancies complicated by a short rib-polydactyly syndrome that was difficult to categorize. Parents from North of Africa were consanguineous (second degree). The third fetus aborted at 22 weeks' gestation because of abnormalities visualized on sonography, has been radiologically and histologically explored.

He presented shortened ribs with thoracic hypoplasia, short limbs with postaxial polydactyly of hands and feet, cystic hygroma and facial dysmorphisms.

The skeletal changes observed included shortened ribs and shortened

curved radii, ulnae, tibiae and fibulae

In autopsy, multiple visceral abnormalities of major organs such as bilateral polycystic kidney and intestinal malrotations were detected. Based on radiological criteria and the pattern of associated abnormalities, short rib-polydactyly syndrome type IV or Beemer-Langer type was retained as diagnosis. The differential diagnosis of this entity is discussed.

#### P01.143

##### **Ellis-Van Creveld syndrome, with bilateral sensory-neural hearing loss: report of a case and literature review**

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Ellis-Van Creveld syndrome is a constellation of chondral, ectodermal and cardiac defects. It is a rare autosomal recessive syndrome with variable expression. This syndrome is also known as chondroectodermal dysplasia and mesoectodermal dysplasia. The main features are short stature, short ribs, polydactyly, dysplastic fingernails and teeth, accompanied by heart defects. We are reporting a 2-year-old girl referred to our genetics center with dwarfism, mesomelic short limbs, narrow thorax, funnel chest, short ribs, oligodontia, oral frenula, post-axial polydactyly of fingers and deafness. Her clinical findings are compatible with Ellis-Van Creveld Syndrome. We believe that this is the first Ellis-Van Creveld Syndrome with sensory-neural hearing loss.

#### P01.144

##### **Skeletal dysplasia with amelogenesis imperfecta- report of a third family**

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Platyspondyly is a frequent feature in skeletal dysplasias, but an association with amelogenesis imperfecta has only been reported twice. Verloes and colleagues (1996) described two siblings of Moroccan origin with consanguineous parents, who had mild growth retardation, platyspondyly, dysplastic femoral necks, amelogenesis imperfecta and oligodontia. They proposed their case to be a new subtype of brachyolmia with amelogenesis imperfecta. A third case was described in a separate paper (Houlston et al, 1994). We present three additional cases from a sibship of five. The affected children, two girls and a boy of 16, 9, and 12 years of age are non-dysmorphic and of normal intelligence. They have short trunk short stature (-5SD, 3-10 centile and respectively -3SD) with platyspondyly and oligodontia. The oldest girl has an S shape scoliosis mid-thoracic to lumbar spine that required surgery and bilateral coxa valga. The other two siblings have osteopenic bones and lower thoracic-lumbar scoliosis. Recurrent dental abscesses were noted and a subsequent diagnosis of amelogenesis imperfecta was confirmed by dental pathology. The parents first cousins of Pakistani origin are healthy and of normal stature. We provide further evidence for a new AR previously proposed condition. Known genes involved in amelogenesis imperfecta (including AR forms) do not explain concurrent skeletal abnormalities.

#### P01.145

##### **From a multimalformed baby to a new skeletal dysplasia**

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In a number of cases skeletal dysplasias (SD) can be associated with other malformations not related to the bone system, but usually the diagnosis of SD is early after birth. In this presentation we report a case of a multimalformed boy, firstly thought representing a VACTERL association, which afterward revealed an unusual skeletal dysplasia. The infant was born at term by caesarean section weighting 2.130 g, 46 cm height, and HC of 34 cm. Prenatal ultrasonography evaluation showed oligohydramnios and absence of right kidney. Soon after birth additional minor dysmorphisms and major anomalies were detected: prominent metopic suture, up-slanting palpebral fissures, bilateral epicanthal folds, ulnar deviation of 4<sup>th</sup>-5<sup>th</sup> fingers, syndactyly of the 2<sup>nd</sup>-3<sup>rd</sup> toes, prominent calcaneus, esophageal atresia with distal fistula, mild ventricular dilatation with tricuspid insufficiency and pulmonary hypertension, dilated bile ducts, and moderate ventricular dilatation sugges-

tive of cerebral atrophy. No skeletal anomalies were observed at this time. Karyotype was normal - 46,XY. Mental retardation was evident in the follow up. At age of 6 years old short stature was evident. Bone age was normal, but the skeletal findings showed findings of SD with spondylo-epi-metaphyseal involvement and also some dyaphyseal lesions. At this time the mother reported two maternal uncles with short stature. As the skeletal lesions were suggestive of spondyloenchondrodysplasia, hydroxyglutaric aciduria investigation was performed, but results were normal. In conclusion, the patient here reported seems to represent a new pattern of SD associated with mental retardation and other no related skeletal anomalies.

#### P01.146

##### **Further evidence for a recessive form of SEMD resembling pseudoachondroplasia in a consanguineous family of Maghrebian origin**

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Pseudoachondroplasia is an autosomal dominant disorder caused by mutations of the COMP gene. In 2005, Spranger et al. described two sibs with a spondyloepimetaphyseal dysplasia (SEMD) resembling pseudoachondroplasia but without a COMP mutation. We have identified a similar skeletal dysplasia in a dizygotic twin and maternal first cousin, born to consanguineous parents of Maghrebian origin. They presented around the age of 1 year with growth failure.

The proband is a 8-year-old girl with short-limb dwarfism (height at -7sd). She is of normal intelligence and has a normal head and face. Clinical features include mild obesity, lumbar hyperlordosis, hyperlaxity of finger and knee joints, metatarsus adductus and waddling gait. Radiographic evaluation reveals mild anterior protrusion of the central aspects of the vertebrae, abnormal pelvis with absent ossification of the femoral epiphyses, shortened tubular bones with small epiphyses and marked metaphyseal changes, and delayed carpal ossification. Similar radiographic changes are observed in her twin brother who in addition to short stature (height at -9sd) has microcephaly and tetraspasticity of unknown origin. A maternal first cousin was referred at the age of 7 years because of short stature (height at -3.5sd) and suspicion of pseudoachondroplasia. Sequence analysis of the COMP gene did not reveal any mutation in this boy.

We believe that the three children in this consanguineous family have the same skeletal dysplasia that resembles pseudoachondroplasia but shows autosomal recessive inheritance. No mutation in the COMP gene was identified suggesting a genetic defect in another gene important in bone growth and development.

#### P01.147

##### **Omani type spondyloepiphyseal dysplasia with cardiac involvement caused by a new missense mutation in CHST3**

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We present three patients with a spondyloepiphyseal dysplasia, brachydactyly and cardiac involvement in a large inbred Turkish family. A genome wide scan using the 250K Affymetrix SNP chip revealed a locus for homozygosity on chromosome 10q23. This interval includes an obvious candidate, Chondroitin 6-O-sulfotransferase-1 (C6ST-1) gene (CHST3), previously shown to be mutated in Spondyloepiphyseal Dysplasia (SED) Omani type. Focusing on CHST3, we amplified the coding region of the CHST3 and identified a homozygous missense mutation (T141M) in the exon 3 of the CHST3 gene in all three of the affected members of the family. Using recombinant C6ST-1, it could be shown that the identified missense mutation reduced the activity of C6ST-1 to 24-29%. This is the second description of SED Omani type further supporting this skeletal dysplasia as a distinct clinical entity. Our patients shared the following features: short trunk stature, progressive spinal involvement, brachydactyly, camptodactyly, irregular-

ity of the endplates of the vertebral bodies, narrowed intervertebral space and small and irregular epiphyses as described by Rajab et al. (Am J Med Genet 2004;126:413) in Omani family. When compared phenotypically to Omani's family, all our patients had also minor facial changes and most importantly they all had a cardiac involvement like mitral, tricuspid and aortal regurgitations, which were not described. The differences in clinical outcome are likely to be due to differences in the nature of the mutation. The original SED Omani mutation resulted in a complete loss of function whereas T141M appears to have residual function.

#### P01.148

#### Tibial Developmental Field Defect is the most Common Lower Limb Malformation Pattern in VACTERL Association

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VACTERL association is one of the most common recognizable patterns of human malformation and has been recently defined as a multiple polytopic developmental field defect. Limb anomalies are a key component of this condition and characteristically reflect perturbation of radial ray development. However, the pattern of appendicular malformations in VACTERL association is wider and includes a broad spectrum of additional and apparently unspecific anomalies. We report on the sporadic case of a 4-10/12-year-old boy presenting with multiple costo-vertebral defects, dextrocardia, bilateral radial ray hypo/aplasia, unilateral kidney agenesis and anal atresia. Homolaterally to the more severe radial ray defect and kidney aplasia, he also has a complex lower limb malformation, consisting of distal tibial aplasia, clubfoot, hallucal deficiency and preaxial polydactyly. Literature review identifies 24 additional patients with VACTERL manifestations and lower limb malformations (excluding cases with isolated secondary deformations). Tibial hypo/aplasia with or without additional tibial field defects, reported in about 2/3 (68%) of the patients, represents the most common finding, while involvement of the fibular ray is rare (20%) and very often accompanies tibial anomalies. The relatively high frequency of tibial ray anomalies in VACTERL patients could easily be explained by the principle of homology of the developmental field theory. Careful search of lower limb anomalies of the "tibial type" is, therefore, indicated in all patients with multiple polytopic developmental field defects.

#### P01.149

#### Hypoplasia of tibia with polysyndactyly (Werner syndrome) is allelic to preaxial polydactyly II (PPD2) and caused by a point mutation in the distant Sonic Hedgehog (SHH) cis-regulator (ZRS)

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Tibial hypoplasia, preaxial polydactyly of hands and feet and/or a five-fingered hand with absence of thumbs was first described by Werner in 1915 (MIM 188770). This autosomal dominant condition is variable and no causative mutations have been described so far. Here we describe an adult Turkish patient with short stature, shortening of forearms, and preaxial polydactyly of hands. His femora are mildly and his lower legs are severely shortened. He had preaxial polydactyly of feet, the supernumerary toe was surgically removed. The clinical diagnosis hypoplasia of tibia with polysyndactyly was established. The patient's father has a preaxial polydactyly of his right hand. We performed mutational analysis in the *Sonic Hedgehog (SHH)* gene and *SHH* regulatory region (ZRS) located in intron 5 of the *LMBR1* gene. A transition (G>A) at position 404 of the ZRS, which was previously reported as the Cuban mutation of the preaxial polydactyly type II (PPD2)-phenotype (Zuriccas et al., 1999; Lettice et al., 2003) was identified in the patient and his father. Single nucleotide substitutions in the ZRS regulatory region, also described in the Hemingway's Cats, operate as gain-of-function mutations that activate *Shh* expression at an ectopic embryonic site (Lettice et al., 2007). Currently, molecular

studies including copy number analysis of the ZRS and *SHH* regions are done in additional families with Werner syndrome, triphalangeal thumb-polysyndactyly syndrome and polysyndactyly type Haas. In summary, we identified the molecular cause of Werner syndrome in our patient and confirmed the previously suggested hypothesis that Werner syndrome is allelic to PPD2.

#### P01.150

#### Fetal phenotype of three cases of campomelic dysplasia harbouring novel mutations of SOX9 gene

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Campomelic dysplasia (CD) is a rare congenital skeletal disorder characterized by bowing of the long bones and a variable association of other skeletal and extraskeletal defects, with or without XY sex reversal.

CD is caused by mutations in the SRY-box 9 gene (SOX9), a dosage-sensitive gene expressed in chondrocytes and other tissues and located at 17q24. The correlation between the campomelic dysplasia genotype and phenotype is still unclear.

In the prenatal period the most characteristic sign of campomelic dysplasia is the shortening and marked anterior bowing of long bones, particularly of femur and tibia. Narrow chest, scoliosis, talipes equinovarus, and flat facial profile are other sonographic features commonly present. Increased nuchal translucency, polyhydramnios, and anomalies of the central nervous, cardiac, and renal systems have also been described.

We report three cases of campomelic dysplasia suspected in the first or second trimester of pregnancy by prenatal ultrasound. The pregnancies were all terminated and the diagnosis of campomelic dysplasia has been confirmed on clinical and radiographic examination of the fetuses.

In the three cases molecular analysis detected three novel mutations in the SOX9 gene which occurred de novo.

The protocol which allowed the specific diagnosis of this rare condition will be presented.

#### P01.151

#### Prenatal diagnosis and management of a patient affected by Kniest dysplasia.

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Patients affected by rare skeletal dysplasias may remain undiagnosed for a long time, until adulthood. In these cases a pregnancy might disclose several unexpected problems and special needs. We present the case of a 28 year old primigravida who was referred to our centre at 17 weeks of gestation with an undiagnosed skeletal dysplasia. Her clinical and radiological examination showed severe dwarfism characterized by spinal curvature anomalies including dorsal scoliosis, lumbar lordosis, platyspondyly, bell-shaped chest, pectus carinatum, drooping ribs, suggesting the diagnosis of Kniest dysplasia (MIM 156550) which is a rare, severe, chondrodysplasia characterized by short trunk and limbs, kyphoscoliosis, midface hypoplasia, severe myopia and hearing loss. The woman was then informed about the recurrence risk and offered ultrasound prenatal diagnosis. Ultrasound examination at 17-18-20 weeks revealed fetal macrocephaly, narrow thorax, shortening and bowing of long bones. Parents decided to continue the pregnancy informed about the clinical features of the fetus. The baby was deliv-

ered at the 33<sup>rd</sup> gestational week for maternal reasons. He experienced severe respiratory distress for four weeks. Diagnosis was confirmed clinically and radiologically. Genomic DNA analysis revealed a new missense mutation in exon 54 (c4339 A>T) of the *COL2A1* gene.

This case demonstrates the relevance of correct diagnosis even in the adult age, when people have the right to know: recurrence risk, risk of pregnancy and delivery and possible neonatal problems of the affected newborn.

#### P01.152

##### More surprises in FGFR2: atypical mutations in Apert syndrome

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Apert syndrome (AS) is one of the most severe craniosynostosis syndromes, characterized by premature fusion of multiple craniofacial sutures and complex syndactyly of the hands and feet. Two heterozygous gain-of-function mutations (Ser252Trp and Pro253Arg) in fibroblast growth factor receptor 2 (FGFR2) are responsible for >98% of AS cases. Here, we have identified different novel mutations in the FGFR2 gene in the last two outstanding Apert patients with unidentified mutation from a cohort of 227 patients. One is a 1.9 kb deletion, removing the entire exon IIIc of the gene and substantial portions of the flanking introns. This is the first large FGFR2 deletion described in any patient with craniosynostosis. The other mutation is a *de novo* *Alu* insertion into the IIIc exon of FGFR2. This is the third identified AS-related *Alu* insertion within 105 bp of sequence, a remarkable enrichment considering that only ~30 new *Alu* insertions have been described in all human diseases. Computational analysis revealed that the inserted *Alu* element belongs to a new subfamily, not previously known to be mobile, which we characterize and term *Alu* Yk. Previous analysis of an AS patient with *Alu* insertion, and a mouse model with an engineered exon IIIc deletion, indicate that both types of mutation are likely to cause AS by driving ectopic expression of an FGFR2 isoform containing the alternatively spliced IIIb exon in mesenchymal tissues. We speculate that the *Alu* insertions, all of which have arisen on the paternal allele, are enriched because of positive selection during spermatogenesis.

#### P01.153

##### Mutations in aggrecan (AGC1) cause Dexter cattle chondrodysplasia

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Lethal "bulldog" chondrodysplasia in Dexter cattle is one of the earliest Mendelian traits described in animals. Affected (homozygous) fetuses display extreme disproportionate dwarfism, a short vertebral column, marked micromelia, short ribs, large head with a retruded muzzle, cleft palate, protruding tongue, and abdominal hernia. Carriers (heterozygotes) show a milder phenotype, having rhizomelic limb shortening in addition to radiographic spinal abnormalities.

Homozygosity mapping in an Australian Dexter cattle pedigree identified the gene *AGC1* as a positional candidate. Homozygous *AGC1* mutations have been shown to cause the lethal chondrodysplasia, cartilage matrix deficiency (cmd) in mice and nanomelia in chicks. Heterozygous *AGC1* mutations cause dwarfism and shortened skeletal elements in mouse and chick, and a spondylo-epiphyseal dysplasia (Kimberley type) associated with severe premature joint and spinal arthritis in humans.

*AGC1* mutation screening revealed a common 4bp insertion in exon 11 (2266\_2267insGGCA) (BD1) and a second, rarer transition in exon 1 (-198C>T) (BD2) that co-segregated with the disorder. We performed allele-specific primer extension analysis of mRNA isolated from chondrocytes of cattle heterozygous for the common insertion (BD1) mutation. This demonstrated that mutant mRNA was subjected to non-

sense-mediated decay, showing only 7% of normal expression, suggesting haploinsufficiency for aggrecan as the pathogenetic basis for the carrier phenotype. Genotyping in Dexter cattle families worldwide has shown that these two mutations account for all cases and segregate fully with the heterozygous or homozygous phenotype.

We anticipate that these Dexter cattle will prove extremely useful as a model for investigating and understanding corresponding human chondrodysplasias and arthritis phenotypes.

#### P01.154

##### Analysis of the Q289P mutation in the FGFR2 gene: populational and computational studies

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The Q289P mutation in the FGFR2 gene was identified in some individuals of a family with clinical features of a Saethre-Chotzen Syndrome (SCS). Considering the variable expressivity of this condition and in this specific family as well, some hypotheses were suggested. The aims of this study were to verify the prevalence of this mutation in different populations and to simulate the effects of this mutation by computational analyses. Three different populations were investigated: 40 individuals with syndromic craniosynostosis and 200 normal controls and all members clinically evaluated from mentioned family. This investigation also includes the search for mutations in hot spots for all individuals with craniosynostosis. Computational approaches were applied to simulate the effects of the mutation in the protein and predict its deleterious potential. Except by the patients in whom the Q289P mutation was previously detected, no more cases were identified. Simulated computational approaches indicated a deleterious potential. We suggested that the Q289P mutation is deleterious, rare and associated to the craniosynostosis phenotype only and not strongly related to the facial and neurological phenotype.

**Key words:** Craniosynostosis; Saethre-Chotzen; FGFR2; Mutation; SIFT; PolyPhen; Grantham

#### P01.155

##### Characterization of two translocation-associated ectrodactyly related loci in distal 2q14.1 and proximal 2q14.2 and the corresponding candidate genes

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Split hand-split foot malformation (SHFM) or ectrodactyly, is a heterogeneous congenital defect of digit formation. The aim of this study is the mapping of the breakpoints and detailed molecular characterization of the candidate genes for an isolated form of bilateral split foot malformation (SFM) and for a syndromic form (holoprosencephaly, hypertelorism, and ectrodactyly syndrome (HGES)) both associated with *de novo* apparently balanced chromosome translocations involving the same chromosome 2q14.2 subband, [t(2;11)(q14.2;q14.2)] and [t(2;4)(q14.2;q35)], respectively. Breakpoints were mapped by fluorescence in situ hybridisation (FISH) using BAC clones. Where possible, these breakpoints were further delimited using PCR fragments as FISH probes. The identified candidate genes were screened for pathogenic mutations by direct sequencing. The SFM associated chromosome 2 breakpoint was localised at 120.9 Mb, between the two main candidate genes, GLI-Kruppel family member GLI2 (*GLI2*) and inhibin beta B (*INHBB*). No clear pathogenic mutation was identified in these. The second breakpoint associated with HGES was mapped 2.5 Mb proximal at 118.4 Mb and the candidate genes identified from this region were the insulin induced protein 2 (*INSG2*) and the homeobox protein engrailed-1 (*EN1*). In conclusion we have confirmed the presence of a new SHFM7 locus in the intergenic region between *INHBB* and *GLI2*. Furthermore, a locus for HGES is proposed 2.5 Mb proximal to the previous one. The molecular mechanism proposed for these congenital anomalies is a sequence of alterations induced by the positional effects introduced by the translocations leading to spatiotemporal misregulated expression of the candidate genes.

**P01.156*****FGFR2 mutations in Turkish patients with craniosynostosis syndrome by DHPLC***

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Fibroblast growth factor receptor 2 (*FGFR2*) gene mutations have been associated with the craniosynostotic conditions of Apert, Crouzon, Pfeiffer, Jackson-Weiss, Saethre-Chotzen, Beare-Stevenson Cutis Gyrata, and Antley-Bixler syndromes in various ethnic groups. Thirty seven unrelated Turkish patients with Apert syndrome (n=8), Crouzon syndrome (n=10), Pfeiffer syndrome (n=3), Saethre/Chotzen syndrome (n=3), and unclassified craniosynostosis (n=13) were screened for mutations in exons IIIa and IIIc of the *FGFR2* gene by polymerase chain reaction, DHPLC and direct sequencing. We established the optimal denaturing High Performance Liquid Chromatography (DHPLC) parameters of each exons using the WAVE Maker Software version 1.6.2. Each anomalous elution peak was then subjected to direct sequencing. Our DHPLC based protocol enabled us to identify the causative mutations in most of the patients, as following, seven of 8 patients with Apert syndrome (S252W,P253R) and six out of 10 patients with Crouzon syndrome (C278F,Q289P,W290R,C342Y), two out of 3 patients with Pfeiffer syndrome (P253R,C342R). We did not detect any *FGFR2* gene mutations in patients with Saethre-Chotzen syndrome or unclassified craniosynostosis patients. The DHPLC based protocol can be used for an efficient, cost effective and reliable mutational analysis of the *FGFR2* gene. In addition, the present study provides a preliminary data in Turkish population, elucidation of the *FGFR2* mutations in patients with clinical features suggestive of especially Apert, Crouzon and Pfeiffer syndrome offers a significant benefit to those families in terms of genetic counseling and prenatal diagnosis.

**P01.157*****Prenatal analysis of dwarfism due to mutations in FGFR3 gene in Spanish population***

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**INTRODUCTION:** Thanatophoric dysplasia (TD), achondroplasia (ACH) and hypochondroplasia (HCH) are skeletal dysplasias with an autosomal dominant pattern. We present the results of FJD skeletal dysplasias cases over eight years in prenatal and miscarriage samples studying mutations in the fibroblast growth factor receptor 3 (*FGFR3*) gene.

**MATERIAL AND METHODS:** Fetal DNA was isolated from amniotic fluids (AF, 15-35 weeks of gestation), villi chorionic samples (CVS, 9-20 weeks) and tissue of abortion (TA, 15-35 weeks). All samples were karyotyped. 5 different PCRs that comprises the more relevant condons (248, 249, 250, 253, 370, 371, 373, 375, 380, 538, 540, 650 and 807) were analysed by automated sequencing analyser. 30 prenatal cases were referred to FJD Laboratory: 18 CVS (60%) and 12 AF (40%).

**RESULTS:** Of the 18 CVS only 2 were positives, and in both cases the pregnant women were also affected of ACH. Of the 12 AF, we obtained 10 negative cases and 2 TD-I+. Main referral (75%) was short limbs and other skeletal anomaly in the present pregnancy. We received 19 TA but only 16 studies because of DNA degraded, with the following results: 1 TD-I; 3 TD-II, 2 aneuploidies.

**CONCLUSIONS:** Molecular analysis of CVS in first trimester is useful when one of the parents is affected due to the 50% of risk. Only severe forms (TD) are detected by ultrasound in second trimester. For miscarriages, is obligatory the fetal karyotype fetal and molecular analysis by sequencing of *FGFR3* gene.

**P01.158*****Postnatal analysis of dwarfism due to mutations in FGFR3 gene in Spanish population***

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**Introduction:** Achondroplasia (ACH), the most common form of human dwarfism (1/10.000-30.000 births), and hypochondroplasia (HCH), a less severe and less frequent condition (1/50.000 births), are inherited in an autosomal dominant manner as other lethal skeletal dysplasias (Thanatophoric dysplasia (TD) type I and II). Mutations in the gene of fibroblast growth factor receptor 3 (*FGFR3*) are known responsible for them.

We present the results about this disorders gather in eight years in our hospital from different geographical origins of Spain.

**Material and Methos:** Patients were all postnatal (neonatal, child and adult people) with a wide range of age. DNA was isolated from blood leucocytes or mouth epithelial cells. 5 different PCRs that comprise the more relevant codons (248, 249, 250, 370, 371, 373, 375, 380, 540, 650 and 807) were analysed by automated sequencing analyser. We also detected the most frequent mutation R380G in ACH phenotype by SNAPSHOT technology.

**Results:** From 77 cases, we obtained 26 ACH+ (33.8%); 7 HCH+ (9.1%) and 1 TD type I+ (1.3%). We found 5 cases with a polymorphic allele (F384L) and 3 cases with a polymorphic allele (G549G) very close to the splice site.

**Cconclusions:** Our strategy for studying all samples is always the same independently the clinical suspicion . Sequencing *FGFR3* is a good practice to detect known and new mutations in individuals affected with different skeletetal dysplasias, especially when few clinical findings are added to the application.

**P01.159*****Functional analysis of osteoporosis pseudoglioma associated missense mutations in LRP5***

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**Background:** Mutations in the low density lipoprotein receptor-related protein 5 gene (*LRP5*) have been associated with high and low bone mass. While homozygous *LRP5* mutations cause osteoporosis-pseudoglioma syndrome (OPPG), characterized by severe osteoporosis and blindness, heterozygous mutations have been associated with reduced bone mass. *LRP5* functions as a plasma membrane receptor in the Wnt signaling pathway. We previously described *LRP5* mutations in patients with OPPG and/or severe osteoporosis. In this study we further analyzed the role of these mutations in Wnt signal transduction and cellular localization.

**Methods:** Mutations were introduced to full length human *LRP5*-pcDNA3.1 expression vector using site-directed mutagenesis. Wnt signal transduction assays were performed in 293HEK cells using a previously published Wnt-induced canonical signaling assay (Ai et al. 2005). Localization studies were performed in COS-1 cells using immunofluorescence staining.

**Results:** Three different missense mutations were identified and selected for further studies. Wnt signaling assays indicated that one of the mutations, R570W in exon 8, completely disrupted Wnt signal transduction. The second mutation, R1036Q in exon 14, resulted in partial disruption of Wnt signaling while the third mutation, R925C in exon 12, did not show any alteration in the signaling assays. Localization studies revealed that the R1036Q and R925C mutant proteins were able to reach plasma membrane where as the R570W could not be detected, suggesting that it might be post-translationally degraded.

**Conclusions:** We were able to show that some *LRP5* mutations directly impair Wnt signal transduction and cellular transportation while other pathogenetic mechanisms are associated with some mutations.

**P01.160****Characterization of the Exostosin-1 (EXT1) promoter***I. Jennes<sup>1</sup>, W. Wuyts<sup>1,2</sup>;*<sup>1</sup>*University of Antwerp, Antwerp, Belgium, <sup>2</sup>University Hospital of Antwerp, Antwerp, Belgium.***Introduction**

Multiple osteochondromas (MO) is an autosomal dominant skeletal disorder characterized by the formation of multiple cartilage-capped protuberances.

MO is genetically heterogeneous and is associated with a mutation in the *EXT1* or *EXT2* tumour suppressor genes. Both genes are ubiquitously expressed and encode proteins that function as glycosyltransferases in the biosynthesis of heparan sulphate.

At present, very little is known about the transcriptional regulation of the *EXT* genes. To elucidate transcriptional regulation of *EXT1*, we isolated and characterized the *EXT1* promoter.

**Methods and results**

Theoretical analysis of the 10 kb upstream of the *EXT1* start codon was performed with promoter prediction programs TSSG, TSSW, FPROM, BDGP, Promoter 2.0 Prediction Server and Web Promoter Scan. This showed presence of a CpG island containing CG and CAAT boxes but no TATA box which, characteristic for a housekeeping gene. Two potential functional promoter regions were identified located respectively ~2.650 bp and ~900 bp upstream of the start codon.

Overlapping PCR fragments of the 10 kb putative *EXT1* promoter region were generated and cloned in the pGL4.7Z Luciferase Reporter Vector. Promoter activity was determined by luciferase assays after transfection in Human Embryonic Kidney (HEK) cells. This situated the actual core promoter within the fragment containing the predicted sequence ~900 bp upstream of the start codon. Further fine mapping of the core promoter and potential regulatory sites was achieved by generating additional subclones, which were subsequently analyzed for promoter activity and analyzed for protein binding capacities. This allowed identification of *EXT1* promoter binding proteins.

**P01.161****Mutation screening for autosomal recessive malignant osteopetrosis***S. Akbaroghi<sup>1</sup>, T. Majidizadeh<sup>1</sup>, M. Dehghan Manshadi<sup>1</sup>, M. Rostami<sup>1</sup>, M. Nataeghi<sup>1</sup>, M. Sanati<sup>2</sup>, M. Houshamand<sup>2,1</sup>;*<sup>1</sup>*Special Medical Center, Tehran, Islamic Republic of Iran, <sup>2</sup>National Institute of Genetic Engineering & Biotechnology, Tehran, Islamic Republic of Iran.*

Osteopetrosis is a congenital disorder characterized by defective or absent osteoclasts, the cells that break down bone. In healthy bone, a balance is achieved between the production of bone by osteoblasts, and the break down of bone by osteoclasts. In osteopetrosis, osteoclasts don't function normally, and the production of bone by osteoblasts leads to bones that are abnormally dense and brittle. Osteopetrosis may result from conditions which interfere with the production of osteoclasts and their ability to remove bone.

Several forms of osteopetrosis exist. The most common form is autosomal dominant. The most severe form of osteopetrosis is termed infantile osteopetrosis, as affected individuals usually have difficulties soon after birth, inherited in an autosomal recessive pattern. The disease is very rare, with about 1/300,000 children having severe osteopetrosis. The candidate genes for Osteopetrosis are *TCIRG1*, *CLCN7* genes. The spectrum of *CLCN7*-related osteopetrosis includes infantile malignant *CLCN7*-related autosomal recessive osteopetrosis, intermediate autosomal and autosomal dominant osteopetrosis type II. Mutations of the *TCIRG1* gene are the most frequent cause of AR osteopetrosis. *TCIRG1* encoding the a3 subunit of the vacuolar proton pump is located on chromosome 11q13 consists of 20 exons.

In present study DNA was received from a consanguineous couple who had 1 affected child from who no sample was available and they were referred us for screening the problem if any, in the fetus. For each sample we performed genetic analysis of hot spot axons of *TCIRG1* by direct sequence analysis. We reported a new mutation in *TCIRG1* gene.

**P01.162****A novel homozygous *COLL11A2* deletion causes a C-terminal protein truncation without mRNA decay in a Turkish patient***O. Z. Uyguner<sup>1</sup>, H. Kayserili<sup>1</sup>, G. Guven<sup>1</sup>, M. U. Emiroglu<sup>1</sup>, N. Baserer<sup>1</sup>, B. Wollnik<sup>2</sup>;*<sup>1</sup>*Istanbul University, Istanbul, Turkey, <sup>2</sup>University of Cologne, Cologne, Germany.*

Otospondylomegaepiphyseal dysplasia (OSMED, OMIM 215150) is a rare autosomal recessive disorder of bone growth and development that results in disproportionate shortness of the limbs with abnormally large knees and elbows, severe hearing loss, and distinctive facial features presenting mid-face hypoplasia, depressed nasal bridge with anteverted nares. Cleft palate and micrognathia are also the common findings. The phenotype is very similar to non-ophthalmic Stickler or Stickler Type III (OMIM 184840), which displays more subtle signs. Recessive mutations on collagen peptide coding gene, *COL11A2*, are responsible for OSMED while dominant mutations are associated with Stickler Type III. Furthermore, mutations in the *COL11A2* gene have been also found in patients with isolated cleft palate, Robin sequences, micrognathia, and non-ophthalmic Stickler syndrome. In this study, two cousins with OSMED were clinically assessed and mutations testing on patient's cDNA identified the novel homozygous c.2763delT in exon 38 of the *COL11A2* gene. The deletion which causes a frame shift after position 425 and a premature stop after additional 62 amino acids (p.P425PfsX62) was confirmed on genomic level in both patients and was not found in 200 ethnically matched control chromosomes.

**P01.163****Mutations of the Sequestosome 1 gene associated with Paget's disease in patients from Salamanca, Spain***E. Corral Moro<sup>1</sup>, L. Corral Gudino<sup>2</sup>, J. García Aparicio<sup>2</sup>, S. Ciria Abad<sup>1</sup>, N. Alonso López<sup>1</sup>, J. del Pino Montes<sup>2</sup>, R. González Sarmiento<sup>1</sup>;*<sup>1</sup>*Unidad de Medicina Molecular-Departamento de Medicina, Universidad de Salamanca, Salamanca, Spain, <sup>2</sup>Servicio de Reumatología, Hospital Universitario de Salamanca, Salamanca, Spain.*

Paget's disease of bone (PDB) is a common condition characterized by focal abnormalities of increased bone turnover.

Mutations in the sequestosome 1/p62 gene (*SQSTM1*) are associated with Paget linked to the 5q35.

Mutations described to date cluster within the C-terminal ubiquitin-associated (UBA) domain of p62 and patients with truncating mutations of p62 in or close to the UBA domain generally suffer more severe disease than those with missense mutations.

We investigated a cohort of 20 familial PDB patients for sequestosome mutations.

We identified three mutations within exon 8: P392L, M404T, previously reported by other groups, and A426V, a novel missense mutation. All three of these mutations affect the ubiquitin-associated (UBA) domain of the protein which is involved in the ubiquitin binding.

Moreover, analysis of exon 6 detected a G to C substitution at position +822, resulting in a glutamic acid to aspartic acid substitution at codon 426. This mutation affects the PEST sequence, important for the span life to the protein. Actually, more studies are under way to examine the effect of his mutation.

**P01.164****Mutations, duplications and deletions, upstream, downstream and overlapping the *SHOX* gene in Leri-Weill dyschondrosteosis or short stature patients of the Balearic islands***J. Rosell<sup>1</sup>, J. Ferragut<sup>2</sup>, M. Caimari<sup>2</sup>, N. Govea<sup>1</sup>, M. Bernues<sup>1</sup>, A. Perez-Granero<sup>1</sup>, D. Heine-Suñer<sup>1</sup>;*<sup>1</sup>*Genetics Hospital Universitari Son Dureta, Palma de Mallorca, Spain, <sup>2</sup>Paediatrics Hospital Universitari Son Dureta, Palma de Mallorca, Spain.*

The *SHOX* gene is located on the pseudoautosomal regions (PAR1) of the X and Y chromosomes. Mutations and deletions of *SHOX* have been shown to be associated with Leri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS). Recently, it has been shown that deletions downstream within the PAR1 region and that do not include the *SHOX* gene can be also associated with LWD. We have studied a total of 120 ISS and LWD patients and their families from the Balearic islands population. The presence of deletions has been determined using microsatellites that are located upstream and/or MLPA (SALSA 18C) which can detect downstream deletions. Mutations have been

analysed by sequencing exons 1 to 6A. We have found deletions, duplications or mutations in a total of 10 families. Three of these families harbour mutations in exon 4, four families had downstream deletions, and three carry deletions overlapping the *SHOX* gene. The remaining family had a duplication upstream of the *SHOX* gene as the sole trait. Interestingly, one of the families with a downstream deletion had also a 5' duplication of *SHOX* up to exon 3 that segregated independently. Deletions and duplications show different breakpoints in each family supporting the idea that there is no main deletion breakpoint hotspot. This work has been supported by the grant FIS 05-1585 from the Instituto de Salud Carlos III from the Spanish ministry of Health.

#### P01.165

#### Detection and characterisation of partial *SHOX* deletions in patients with Léri-Weill dyschondrosteosis (LWD), Langer mesomelic dysplasia (LMD) and Idiopathic Short Stature (ISS)

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*SHOX* is located in the pseudoautosomal region 1 (PAR1) of the X and Y chromosomes. Mutations in *SHOX* or in the downstream PAR1 have been shown to be the cause of Léri-Weill dyschondrosteosis (LWD), Langer mesomelic dysplasia (LMD) and idiopathic short stature (ISS). We routinely perform deletion screening of *SHOX* and the downstream PAR1 in LWD, LMD and ISS patients using MLPA. Microsatellite markers and SNPs are then utilized for further characterisation in specific cases.

During our screening, we identified nine partial *SHOX* deletions (five LWD, one LMD and three ISS). The deletions were all of variable size, ranging from the deletion of a single exon to multiple exons. Polymorphisms in the ligation sites were excluded in cases where deletions only included one exon. A common 5' breakpoint region in intron 3 was observed in five patients. Three patients also share the 3' limit in intron 2 and two more in the 3'UTR. Deletion breakpoints are currently being further delimited by fine-tiling CGH arrays and subsequently amplification across the breakpoints.

MLPA is an accurate, rapid and economic technique to detect complete and partial *SHOX* deletions as well as downstream PAR1 deletions. FISH and microsatellite analysis cannot accurately detect or delimit this class of deletions. Our results show that, although the partial deletions of *SHOX* are variable in size, intron 2 and 3 appear to be hotspots for breakages.

#### P01.166

#### Mosaicism of the *SHOX* gene in a serie of Spanish patients with short stature

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The *SHOX* gene, located at the pseudo-autosomal region (PAR1) of the X (Xp22) and Y (Yp11) chromosomes codifies for a transcription factor implicated in the regulation of skeletal growth. Deletions or mutations in *SHOX* may lead to haploinsufficiency of this gene and sometimes is associated with Turner syndrome, Leri-Weil dyschondrosteosis (LWD), Langer type mesomelic dysplasia (LMD) and idiopathic short stature (ISS).

**PATIENTS AND METHODS:** Using STRs (microsatellites, repeated sequences in tandems distributed in the DNA) we evaluated the copy number of *SHOX* and/or their different alleles in 340 patients with short stature. The peak areas of both alleles were evaluated by means of the ratio between both alleles and then compared and normalized with normal controls. Calculation was expressed as a percentage of each allele.

**RESULTS:** We detected mosaicism for *SHOX* in 5 out of 340 patients. Normalization showed a percentage of deletion of 46, 33, 26, 26 and 11% respectively. Karyotypes (100 cells were evaluated for each pa-

tient) were normal in all patients.

**CONCLUSION:** Though classical haploinsufficiency of the *SHOX* gene shows a heterogeneous phenotypic expression in patients with short stature, patients with mosaicism of this gene should be recognized as a special group of children since the genetic dose of the gene is variable and the final height and evolution is unpredictable.

#### P01.167

#### Microduplication of the long range SHH limb regulator (ZRS) is associated with triphalangeal thumb-polysyndactyly syndrome

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An important player in establishing the anterior-posterior patterning of the limb is the developmental regulator gene *sonic hedgehog* (*SHH*). Previous studies have identified a long range regulator for *SHH* expression in the limb bud residing in a highly conserved non-coding sequence about 1 Mb upstream from the *SHH* gene itself. As shown in mice point mutations within this non-coding regulatory region designated ZRS lead to ectopic expression of Shh in the anterior margin of the limb bud and thus to preaxial extra digits. In humans ZRS point mutations are associated with the triphalangeal thumb and polysyndactyly (TPT-PS, OMIM #174500) phenotype.

In this study we investigated a large pedigree with a variable phenotype of TPT-PS. Although linkage to the *SHH* locus was confirmed sequencing of the ZRS did not reveal point mutations. A subsequent screening by array-CGH detected a microduplication in 7q36.3 comprising the ZRS in an affected individual. The microduplication was confirmed by qPCR in all affected family members. By using a direct sequencing strategy we showed that the duplicated segment is in direct tandem orientation.

In summary we demonstrated that microduplication of the ZRS region in 7q36.3 results in a similar phenotype as caused by point mutation in the limb specific *SHH* regulatory element. Thus, genomic duplications have to be considered as a possible mechanism which leads to disturbance of long-range transcriptional control. The discovery of novel mechanisms of gene regulation, i.e. distant enhancers/repressors and their relevance to human disease if disrupted is a challenging task in the future.

#### P01.168

#### The transcription factor TRPS1 interacts with the RINGfinger ubiquitin ligase ARKADIA

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Mutations or deletions of the *TRPS1* gene on human chromosome 8q24.1 cause the tricho-rhino-phalangeal syndromes (TRPS), which are characterized by craniofacial and skeletal abnormalities. The gene encodes a transcription factor that functions as a repressor for GATA-mediated transcription. The activity of transcription factors is often controlled by post-translational modifications. And in fact, we have recently found that SUMOylation of specific sites within the repression domain (RD) of *TRPS1* regulates its function.

In a yeast-two-hybrid screen we identified two clones encoding amino acids (aa) 352-505 of the 986 aa protein ARKADIA. ARKADIA is a RINGfinger ubiquitin ligase. In mice, Arakdia is known as a key regulator in the TGF-beta pathway by inducing the ubiquitin-dependent degradation of Smad7, SnoN and c-Ski. By using a variety of truncated *TRPS1* and ARKADIA constructs we could narrow down the ARKADIA-binding region within *TRPS1* to the last 100 aa, which includes the RD. ARKADIA appears to interact with *TRPS1* via two different regions, which were also described to enable the interaction of ARKADIA with SMAD7. *TRPS1* is known to be localized within the nucleus. Selective inhibition of the proteasome complex with lactacystin results in cytoplasmic accumulation of *TRPS1* in cells co-transfected with ARKADIA. Furthermore, in luciferase reporter gene assays we could show that ARKADIA decreases the repressional activity of *TRPS1*. Our results strongly indicate an ARKADIA-mediated ubiquitination which induces a cytoplasmic degradation of *TRPS1*.

**P01.169****A variant in the ZRS sonic hedgehog regulatory sequence is associated with triphalangeal thumb and deregulates expression in the developing limb**

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A locus for triphalangeal thumb, variably associated with preaxial polydactyly, was previously identified in the Zone of Polarizing Activity Regulatory Sequence (ZRS), a long range limb-specific enhancer of the Sonic Hedgehog gene (*SHH*) at human chromosome 7q36.3. Here, we demonstrate that a 295T>C variant in the ZRS, previously thought to represent a neutral polymorphism, acts as a dominant mutation with reduced penetrance. We found this variant in 3 independently ascertained probands from southern England with triphalangeal thumb, demonstrated significant linkage of the phenotype to the variant (LOD = 4.1), and identified a shared microsatellite haplotype around the ZRS, suggesting that the probands share a common ancestor. An individual homozygous for the 295C allele presented with isolated bilateral triphalangeal thumb resembling the heterozygous phenotype, suggesting that the variant is largely dominant to the wild type allele. As a functional test of the pathogenicity of the 295C allele, we utilised a mutated ZRS construct to demonstrate that it can drive ectopic anterior expression of a reporter gene in the developing mouse forelimb. We conclude that the 295T>C variant is in fact pathogenic and appears to be the most common cause of triphalangeal thumb in southern England. Depending on the dispersal of the founding mutation, it may play a wider role on the aetiology of this disorder.

**P01.170****Fetal Alcohol Syndrome a phenocopy of Spondylocarpotarsal synostosis syndrome?**

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Spondylocarpotarsal synostosis syndrome is characterized by disproportionate short stature with fusion of vertebrae, carpal and tarsal bones, thoracic scoliosis and pes planus. Other features often described include delayed bone age, epiphyseal ossification delay, cleft palate, dental enamel hypoplasia and renal anomalies. Skeletal manifestations of fetal alcohol syndrome (FAS) include poor growth, delayed bone age, congenital fusion of cervical vertebrae, coalition of the capitate and hamate carpal bones and transverse limb defects. We report a 6-year-old girl born to a 32-year-old mother and a 62 year old father after a pregnancy complicated by alcohol abuse. She presented with short stature, hypotonia, dysmorphic facial features, generalized joint laxity and hyperextensibility and hearing loss. She also had poor visual acuity, extremely high bilateral myopia, pale optic nerves, thin retinal vessels and tigroid retinae. Subsequently, she developed an asymptomatic complete funnel retinal detachment of the right eye. Her skeletal survey revealed normal bone age, fusion of vertebral facet joints of C2-4 and C5-6 with narrowing of these disc spaces but widening of the C4-5 and C6-7 disc spaces, flared ilii, longer proximal than distal long bones, fused capitate and hamate ossification centres and bilateral coxa valga. We postulate that prenatal alcohol exposure can phenocopy spondylocarpotarsal synostosis syndrome. Alternately, this patient's features could represent an overlap of fetal alcohol spectrum disorder with spondylocarpotarsal synostosis syndrome, an extension of the spondylocarpotarsal synostosis syndrome or an undescribed syndrome.

**P01.171****Variable phenotype of Hennekam syndrome**

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Hennekam syndrome is a rare autosomal recessive anomaly characterized by lymphedema, lymphangiectasia, facial dysmorphism and developmental delay. Heart, renal, skeletal anomalies and growth retardation may also be a part of the spectrum. Severity of the syndrome

is due to lymphangiectasia, preferentially intestinal, and mental retardation.

We describe two new unrelated cases of Hennekam syndrome.

The first patient, a 3-year-old girl, is the only infant born of non-consanguineous parents. At birth, she presented facial edema. Lymphedema rapidly extended to legs and feet. Enteropathy appeared during the first year. At 3 years, she presented the facial dysmorphism of Hennekam syndrome and psychomotor development was normal.

The second case, a 36-year-old woman, is the only child of non-consanguineous parents.

At birth, syndactyly of toes was present associated with particular facial features. Later, she developed psychomotor delay with mental retardation and seizures. Lymphedema appeared on the right leg at 3 years and on the left leg at 15 years. Recurrent episodes of diarrhoea were present without enteropathy.

Abdominal cystic lymphangioma were surgically corrected at 30 years. At 36 years, facial dysmorphism resembled that of Hennekam syndrome. She had short hands, hypoplastic thumbs, short feet with bilateral syndactylies. These two observations confirm the variable expressivity of Hennekam syndrome. Lymphedema of the limbs and facial dysmorphys are the only constant features. All other traits, in particular intestinal lymphangiectasia, may be absent.

**P01.172****Novel VEGFR3 missense mutation in a Spanish family with Milroy disease**

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Introduction: Milroy disease, or hereditary lymphedema type 1 (MIM153100), is characterized by lower limb lymphedema usually present at birth. Variability in expression has been reported both within and among families. Mutations in VEGFR3 with resultant dysgenesis of microlymphatic vessels cause this disease. It is inherited in an autosomal dominant manner with incomplete penetrance (85-90%).

Objective: We describe phenotype of Milroy disease in two-generation Spanish family. Molecular analysis of VEGFR3 gene has been performed.

Clinical description: A girl was referred at 2 years of age because of bilateral swelling of feet since birth, more evident on the right foot, which partially decreased during the first year of life. She had dysplastic 2-4th toenails. She was the first child of a young and non-consanguineous couple. Her father referred swelling of feet at birth which disappeared progressively during childhood. He had dysplastic toenails. Soon after a sister was born with swelling of feet and distal part of limbs as well. She is now 4 years old and the lymphedema is stable; dysplastic nails are not present. The son of a father's asymptomatic sister has also been born with bilateral swelling of feet. Analysis of VEGFR3 gene revealed a heterozygote mutation c.3056T>C in exon 22 in the two girls and their father.

Conclusions: 1- We describe a novel VEGFR3 mutation in exon 22 causing Milroy disease. 2- Clinical variability and incomplete penetrance is present in this family.

**P01.173****VEGFR3 mutation frequency in Milroy disease and other primary lymphoedema**

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Milroy disease (Hereditary Lymphoedema type I) is a congenital onset primary lymphoedema with autosomal dominant inheritance. Mutations in the gene, vasculo-endothelial growth factor receptor, VEGFR3 (*FLT4*), are known to cause Milroy disease, but there is uncertainty about the prevalence of VEGFR3 mutations in patients with primary lymphoedema and more specifically in those with a phenotype that resembles Milroy disease. This study addresses this issue and thereby delineates the Milroy disease phenotype. Fifty-four patients with primary lymphoedema were analysed for mutations in VEGFR3. Patients were divided into four groups: Typical Milroy disease with family history (group I), typical Milroy disease with no family history (group II), atypical Milroy disease (group III), and complex primary lymphoedema (group IV). Results demonstrated that with rigorous phenotyping

the likelihood of detecting *VEGFR3* mutations is optimised. Mutation prevalence is 72% in typical Milroy patients with a family history (group I) and 64% if positive family history is not a diagnostic criterion. A positive family history is not essential in Milroy disease. The likelihood of detecting *VEGFR3* mutations in patients who have a phenotype which is not typical of Milroy disease is very small (<5%). For the 22 mutation positive patients, 14 novel *VEGFR3* mutations were identified, two of which were in exon 22 and one in exon 17, confirming that these exons should be included in *VEGFR3* analysis.

#### P01.174

#### **Microcephaly, lymphedema, chorioretinal dysplasia syndrome (MLCRD) and atrial septal defect (ASD) further delineation of a rare syndrome**

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Combinations of microcephaly and lymphoedema, as well as microcephaly and chorioretinopathy, have been well described in the past. It has been later suggested these two entities represent a single entity: lymphoedema, microcephaly, chorioretinopathy syndrome (MLCRD OMIM 152950). To date, less than 30 patients have been reported in the literature. This entity is characterized by microcephaly, lymphoedema of the limbs, chorioretinal dysplasia, and normal or near-normal intelligence. The inheritance can be autosomal dominant, or recessive. A few sporadic instances have also been reported. Although this condition is thought to be a single genetic entity with variable expression, only three cases were previously described in the literature with cardiac anomaly.

We describe here another case of MLCRD with a cardiac anomaly. A six months male infant has been first referred to our clinic because of microcephaly and lymphedema. Further evaluation revealed chorioretinal changes, severe myopia and atrial septal defect (ASD). Brain MRI was normal. He is the first child of healthy and unrelated couple with unremarkable medical history, except for nonsyndromic familial microcephaly in the mother's family.

At the age of one year he came for a regular follow up to our clinic. The infant developed normally and achieved the expected motor, mental and social milestone for his age.

This case further emphasizes the inter- and intra-familial variable expressivity of MLCRD.

#### P01.175

#### **Neurofibromatosis-Noonan like Syndrome/Watson Syndrome-the syndromes of the same mitogen-activated protein kinase (MAPK) pathway.**

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Since 1967, when G.H. Watson described first three families with a condition characterized by café au lait spots, pulmonary valvular stenosis, short stature and dull intelligence-there have been only few more patients diagnosed with this disease. The authors report a 11-month-old child manifesting the symptoms, which are in the picture of Neurofibromatosis-Noonan like /Watson syndrome: café au lait spots, pectus abnormality, pulmonary stenosis. Additionally, magnetic resonance imagining of the head demonstrated the lesion, diameter about 4mm change, situated in the capsula interna on the border with the posterior part of thalamus which is possibly hamartoma. Significant is, as well, the family history- the father of the child has phenotype of Neurofibromatosis-Noonan like /Watson Syndrome, although, his condition was diagnosed as neurofibromatosis type I in childhood. The authors analyse the clinical phenotype, overlapping characteristic features of above mentioned syndromes and discuss the molecular basis of this phenomenon.

#### P01.176

#### **Noonan syndrome and marfanoid habitus in a patient with SOS1 mutation**

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We report the observation of a 24-year-old male patient exhibiting features suggesting Noonan syndrome (mild mental retardation, low-set hair, hypertelorism, bilateral ptosis, dysplastic low-set ears, and webbed neck), and others compatible with Marfan disease (height at 1m83, joint hyperlaxity, arachnodactyl, pectus carinatum, severe scoliosis, myopia). Cardiac ultrasound examination was normal. Familial stature was tall (mother 1m73, father 1m83, and brothers 1m95 and 2m). Blood standard karyotype was normal. Noonan syndrome was confirmed by molecular analysis of *SOS1* gene (heterozygous mutation 1654 A>C (R552G)). However Marfan disease was not documented with a negative screening of *FBN1* gene. We discuss the hypothesis of a pleiotropic effect of the *SOS1* mutation, the influence of the familial stature, and the eventualty of two concomitant independent diseases.

#### P01.177

#### ***SOS1* and *PTPN11* mutations in five cases of Noonan syndrome with multiple giant cell lesions**

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The five patients have typical features for Noonan syndrome. Congenital heart diseases were diagnosed during gestation or after birth. Radiograph and CT scan showed unilateral or bilateral mandibular radiolucencies characteristic of cherubism for five patients with cherubic appearance or incidentally discovered at systematic radiographic examination. The other patient presented typical imaging and histopathological signs of a left ankle pigmented villonodular synovitis. Three of the five patients had short stature and three had learning disabilities. They show two distinct phenotypes: Noonan with cherubism and Noonan with pigmented villonodular synovitis, demonstrating that both clinical phenotypes are not *PTPN11*-specific anomalies and represents a rare complication of overexpression of the RAS/MAPK signaling pathway. We discuss the clinical overlap between Noonan syndrome, Noonan like-Multiple Giant Cell syndrome, "true" cherubism and pigmented villonodular synovitis. The two latter terms should be used only when multiple giant cell lesions occur without any other evidence of Noonan syndrome. Noonan like-Multiple Giant Cell syndrome previously regarded as an entity distinct from Noonan syndrome is more likely as a phenotypic variation within the Noonan spectrum.

#### P01.178

#### **Clinical study of a family with Noonan Syndrome (NS) transmitted from mother to daughter**

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Noonan Syndrome (NS) is an autosomal dominant disease, with mutation inherited especially from mother, characterized by typical facial dysmorphism, short stature, heart defect, mental retardation and he-

matological anomalies. Almost 50% of patients with NS (sporadic or familial) carry an missense mutation of PTPN11 gene (12q24), while other genes ( KRAS and SOS1) play a minor role in the molecular pathogenesis of the disease.

We present a familial case, mother and daughter, with NS diagnosed by used a scoring system based by clinical signs.

The diagnosis was performed first in daughter, which was identified at birth. She presented polyhidramnios, nuchal oedema and premature born. The patient was reevaluated of two months age. Clinical and paraclinic evaluation revealed the major features of NS: feeding difficulties with failure to thrive, short stature, typical facial dysmorphies (microcephaly, broad and high forehead, down-slanting palpebral fissures, hypertelorism, epicanthic folds, micrognathia, low-set posteriorly rotated ears), short neck with excess nucal skin, pectus excavatum, Hypertrophic Obstructive Cardiomyopathy (HOMC) with asymmetrical septum hypertrophy and neuromotor retardation. Other feature was umbilical hernia. She had anemia, but no thrombocytopenia or other coagulation defect. Clinical genetic examination of mother (N.C.M.), 31 years old, after delivery revealed the phenotype of NS (facial typical dysmorphisms, pterygium colli, pectus carinatum superior and excavatum inferior), pulmonar stenosis and mental retardation. The analysis of pedigree showed the absence of other cases in family. In conclusion, we emphasize, the importance of the clinical features for diagnosis, leading further to an appropriate management and for according genetic counseling.

#### P01.179

#### Noonan and Cardio-facio-cutaneous syndromes: Two Clinically and Genetically Overlapping Disorders

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Noonan and Cardio-facio-cutaneous (CFC) syndromes are related disorders associated with dysregulated RAS-MAPK signalling. Noonan syndrome (NS) is associated with mutations in the genes PTPN11, SOS1, KRAS and RAF1. The genetic aetiology of the clinically overlapping CFC syndrome was recently assigned to four genes, BRAF, KRAS, MEK1 and MEK2. Here, we present a comprehensive mutation analysis of BRAF, KRAS, MEK1, MEK2 and SOS1 in 31 unrelated NS/CFC patients without mutations in PTPN11. Mutations were identified in 8 patients, in whom we also present a detailed clinical investigation. Seven of the mutations were identified in patients with CFC diagnoses (2 in BRAF, 1 in KRAS, 1 in MEK1, 2 in MEK2 and 1 in SOS1). Two mutations were novel: MEK1 E203Q and MEK2 F57L. The SOS1 E433K mutation, identified in a patient diagnosed as CFC, has previously been reported in patients with NS. In one NS patient, we also identified a BRAF K499E mutation, previously reported in patients with CFC. We thus suggest involvement also of BRAF in the pathogenesis of NS.

Taken together, our results indicate that the molecular and clinical overlap between CFC and NS is more complex than previously suggested and that the syndromes might even present as allelic disorders. Furthermore, we suggest that the diagnosis should be refined to, e.g., NS-PTPN11-associated or CFC-BRAF-associated after the genetic defect has been established since this may have an impact on prognosis and treatment of the patients.

#### P01.180

#### Clinical and molecular studies in 95 polish patients with noonan syndrome

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Noonan syndrome (NS, OMIM 163950) is genetically heterogeneous disorder characterized by facial dysmorphisms, short stature, variety of heart defects (particularly pulmonary stenosis), chest deformations, pterygium colli, lymphatic dysplasia, cryptorchidism and psychomotor retardation. Heterogeneous mutations in PTPN11 gene are responsible

for 29-60% of NS cases. The aim of our study was to estimate the frequency of PTPN11 gene mutations in Polish patients with NS. Results of clinical and molecular studies the group of 95 probands are reported. Detailed clinical evaluation, including family pedigree, dysmorphic features, pre- and postnatal development and congenital malformations is presented. Direct sequencing analysis of the 15 exons of PTPN11 gene was performed and mutation was found in 32 (35%) of the probands. The most frequent among 15 identified missense mutations were: 1510A>G and 922A>G. In 9(28%) patients mutation was familiar and in 77% of cases was maternal in origin.

All patients with PTPN11 mutation demonstrated typical NS phenotype. Pulmonary valve stenosis, atrial septum defect, coagulation abnormalities, mild mental retardation, short triangular face with pointed chin were statistically the most frequent in patients with PTPN11 mutation. Statistically significant correlation of pulmonary stenosis with 922A>G and 1510A>G mutation was found. Heart defect was present in all probands with PTPN11 mutation, but only in 66% of their parents.

We concluded that probably another mutations within SOS1, KRAS and BRAF genes involved in the RAS-MAPK pathway may be responsible for NS in subset of presented patients without PTPN11 mutation. Further investigations are planned to explain NS phenotype in these patients.

#### P01.181

#### SOS1 mutation as a cause for Noonan syndrome type 4: A report of two patients

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Noonan syndrome (NS) is an autosomal dominantly inherited disease characterized by mental retardation (25%), short stature, pterygium colli, congenital heart defect and hypogonadism. Approximately 50% of cases are caused by mutations in the PTPN11 gene on chromosome 12q24.1. Recently, Roberts and Tartaglia (2007) found mutation in the SOS1 gene on chromosome 2p22 to be responsible from %20 of NS who had ectodermal abnormalities but normal development. This subtype called NS4 which share many clinical features with a group of developmental disorders including Costello and CFC (Cardiofaciocutaneous) syndrome. Our first patient was a 7 month old male and the second was 45 days old girl who had coarse face, sparse hair and eyebrows and skin loss. Valvular pulmonary stenosis was shown in echocardiography at the first patient and atrioventricular channel defect and pulmonary hypertension at the second. One of our patients had signs of hyperkeratotic skin too. SOS1 mutation were detected on exon 6; c.797C>A heterozygous, p.T266K and on exon 16 c.2536G>A heterozygous p.E846K in patient 1 and patient 2 respectively. Pulmonary stenosis was found more frequently in patients with SOS1 mutations than PTPN1 mutations and atrial septal defect was relatively rare in affected individuals with SOS1 mutations. Thus clinical examination can be indeterminative. It is important to remember to identify SOS1 gene on patients who are free of BRAF, KRAS, MEK 1, 2 mutations that are responsible from the etiology of CFC syndrome.

#### P01.182

#### Prenatal images and evolution to cutis verticis gyrata in a newborn with Noonan syndrome

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Cutis verticis gyrata (CVG) is a rare defect, characterized by skin ridges and furrows forming cerebriform folds. Its primary form is associated with neuropsychiatric disorders or genetic syndromes, such as Turner and, more rarely, Noonan. Secondarily, CVG may be due to a local process or a systemic illness.

**Objective:** To show the prenatal images of CVG in a fetus, and its evolution until birth, when Noonan syndrome was diagnosed.

**Case report:** The propositus was the third child of a young and healthy couple. Pregnancy was uneventful until 18 gestational weeks, when an ultrasound (US) scan revealed a cystic hygroma, hydrothorax and a fluid collection at the cephalic pole, which first suggested an underlying

skull defect, until bony tissue between the intracranial structures and the overlying lesion could be identified. At 22 gestational weeks, the mentioned fluid collection had disappeared, leaving a residual scalp thickening and an enlarged nuchal fold. Fetal karyotype by amniocentesis was normal (46,XY). At birth, the infant showed a Noonan syndrome phenotype and a CVG.

**Discussion:** CVG is possibly due to an initial scalp lymphedema and its further resolution, and should be distinguished from a cephalocele. This is the third description of a patient with Noonan syndrome and CVG, and the second describing a fluid collection at the cephalic pole that progressed to CVG. The observed images and their evolution are of clinical importance, because they can strengthen the suspicion of Noonan syndrome prenatally.

#### P01.183

#### Gain-of-function *RAF1* mutations cause Noonan and LEOPARD syndromes with hypertrophic cardiomyopathy

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Noonan and LEOPARD syndromes (NS and LS) are developmental disorders with overlapping features including cardiac abnormalities, short stature and facial dysmorphisms. Increased RAS signaling due to *PTPN11*, *KRAS* and *SOS1* mutations cause approximately 60% of NS cases, while *PTPN11* mutations cause approximately 90% of LS cases. Here, we report that 18 of 231 NS and two of six LS patients without mutations in known genes have missense mutations in *RAF1*, which encodes a serine/threonine protein kinase that activates MEK1 and MEK2. Most mutations altered a motif flanking Ser259, a residue critical for *RAF1*'s autoinhibition through 14-3-3 binding. *RAF1* mutations in two hotspots were strongly associated with hypertrophic cardiomyopathy (HCM; 95% vs. 18% of all NS). Ectopically expressed *RAF1* mutants from HCM clusters had increased kinase activity and enhanced ERK activation, while non-HCM-associated-cluster mutants were kinase impaired. Our findings further implicate increased RAS signaling in pathological cardiomyocyte hypertrophy.

#### P01.184

#### SOS1 mutation in a CFC patient

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After the CFC syndrome was originally published in 1986 it was continuously debated whether it can be separated from the Noonan syndrome. Molecular genetic studies have shown that at least one gene in the RAS pathway, KRAS can cause both syndromes. When SOS1 was described to be the second most common gene in Noonan it was emphasized that it usually does not cause the CFC syndrome. We describe a 4-y-o boy with SOS1 exon 6 missense mutation c.797C>A (p.T266K), earlier reported by Roberts et al in 2007 in a Noonan patient. Parental studies were normal. He was born at after 37 weeks of pregnancy complicated by fetal hydronephrosis and polyhydramnios with birth weight 4000g, length 51 cm and OFC 36,5 cm . There was oedema, opistothonus, poor feeding, cryptorchidism, and umbilical hernia. He has pulmonary and mild aortic stenosis, ASD secundum, and hypertrophy of the ventricular septum. His growth is on the -1.2 SD curve and OFC +1.5 SD. He started to walk at 18 months but is not yet able to run. He spoke the first words at 3,5 years and mainly uses sign language. He has melatonin treatment for poor sleeping patterns. Dysmorphic features are compatible with the CFC syndrome with bitemporal constrictions, sparse hair, absent eyebrows, dystrophic nails

and hyperkeratotic eczema on face and legs. SOS1 mutation found in this patient with CFC phenotype further emphasizes the need of phenotype-genotype correlation studies in SOS1 mutation patients.

#### P01.185

#### Improved Mutation Detection in the Polygenic Disorders Noonan Syndrome and LEOPARD Syndrome

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Noonan syndrome (NS) is a common autosomal dominant multiple congenital anomaly syndrome exhibiting short stature, heart defects and facial dysmorphisms. LEOPARD syndrome (LS) is a rare autosomal dominant multiple congenital abnormality syndrome. The acronym LEOPARD defines the common features: multiple Lentigines, ECG conduction abnormalities, Ocular hypertelorism, Pulmonary stenosis, Abnormal genitalia, Retardation of growth and sensorineural Deafness. Defects in the RAS-MAPK pathway have been implicated in both syndromes.

Since 2001 missense mutations in the *PTPN11* gene have been recognised as causative in approximately half of NS cases and the majority of LS cases. We established a service in 2002 to analyse exons 3 and 8 of this gene which contain ~80% of known NS mutations. Since 2005 we have screened all exons in which mutations had been identified and thus allowed molecular confirmation of LS.

Mutations in the *SOS1*, *RAF1* and *KRAS* genes, which also encode proteins in the RAS-MAPK pathway, have been identified in 10-20% of *PTPN11* negative NS cases. *RAF1* mutations have also been identified in a proportion of *PTPN11* negative LS cases. We have set up a diagnostic service to analyse the known mutation-containing exons of *SOS1* and *RAF1* and all exons of *KRAS*. Testing involves a dHPLC pre-screen followed by bidirectional sequencing of any variants. We have identified 12 pathogenic mutations in *SOS1* and *RAF1* in our initial cohort of 110 *PTPN11* negative cases referred for NS/LS. This analysis is now integrated with the *PTPN11* screen to facilitate a comprehensive molecular investigation of NS and LS referrals.

#### P01.186

#### Molecular analysis of the *PTPN11* (protein-tyrosine phosphatase, nonreceptor-type 11) gene in Estonian patients with Noonan syndrome

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Noonan syndrome (NS; OMIM# 163950) is a relatively common (1:1000-2500) autosomal dominant disorder characterized by proportional short stature, facial dysmorphisms and cardiac anomalies. Missense mutations of *PTPN11* gene have been documented in about 50% of NS cases, while molecular lesions of other genes of the RAS pathway (*KRAS*, *SOS1*, *RAS1*) play a minor role in the molecular pathogenesis of the disease.

Study group consisted of 10 probands (age 1-36 y; 5 males/5 females), 4 of which were familial cases. All patients had the typical face and inclusion diagnostic criteria of NS proposed by van der Burgt (1994). All of the patients had a normal karyotype. Sequence analysis was performed for five coding *PTPN11* exons 3, 4, 7, 8, 13 and their flanking regions. Mutation analysis of the *PTPN11* hot spot coding exons revealed two different heterozygous missense mutations in two unrelated families. A transition A1510G in exon13 for family NS-VI, which predicts the Met504Val substitution, was described previously (Tartaglia, 2001), resided in the PTP domain of SHP-2 protein. A172G tran-

sition was identified in the exon3. This change predicts the Asn58Asp substitution in family NS-X, and affects the N-SH2 domain of SHP-2. Patients with *PTPN11* disease related mutations had characteristic phenotypes: a proband from NS-VI family presented short stature, dysmorphic facial features and atrial septal defect; a proband from NS-X family had short stature, pulmonary valve stenosis and dysmorphic facial features. Future studies involving clinical and genetic investigations are necessary to correlate genotype-phenotype expression. Supported by Estonian Science Foundation grant GARMP6573.

#### P01.187

#### **PTPN11 gene analysis in a sample of Mexican patients with Noonan syndrome**

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Noonan syndrome (NS) is a dysmorphic syndrome characterized by hypertelorism, low-set posteriorly rotated ears, short stature, a short neck with webbing or redundancy of skin, cardiac anomalies, epicanthic folds and motor delay. NS may occur on a sporadic basis, with a predominance of paternal origin, or in an autosomal dominant inheritance with a predominance of maternal transmission. NS is clinically similar to the Turner syndrome. More than 50% of patients with NS have mutations in the *PTPN11* gene. Most of the mutations are recurrent and cluster in exons 3, 8 and 13. All the *PTPN11* missense mutations are present in the interacting regions of the amino N-SH2 domain and the phosphotyrosine phosphatase (PTP) domains. It seems that gain-of-function changes are responsible of the phenotype observed in NS. In the present study we analyzed the *PTPN11* gene in a group of 10 patients with diagnosis of NS. DNA genomic from leukocytes was extracted with conventional methods. All exons of the *PTPN11* were amplified and sequenced through polymerase chain reaction and DNA sequencing analyzes. We detected a low frequency of mutations in the *PTPN11* gene in the patients; most patients showed a normal sequence of the *PTPN11* gene. These data indicate that probably *PTPN11* gene mutations are not the most frequent cause of NS in Mexican population.

#### P01.188

#### **Diverse driving forces underlie the occurrence of invariant PTPN11 mutations in Noonan and LEOPARD syndromes**

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Missense *PTPN11* mutations cause Noonan and LEOPARD syndromes (NS and LS), two developmental disorders with pleiomorphic phenotypes. *PTPN11* encodes SHP2, an SH2 domain-containing protein tyrosine phosphatase functioning as a signal transducer. Generally, different substitutions of a particular amino acid residue are observed in these diseases, indicating that the crucial factor is the residue being replaced. For a few codons, only one substitution is observed, suggesting the possibility of specific roles for the residue introduced. We analyzed the biochemical behavior and ligand-binding properties of all possible substitutions arising from single-base changes affecting codons 42, 139, 279, 282 and 468 to investigate the mechanisms underlying the invariant occurrence of the T42A, E139D and I282V substitutions in NS and the Y279C and T468M changes in LS. Our data demonstrate that the isoleucine-to-valine change at codon 282 is the only substitution at that position perturbing the stability of SHP2's closed conformation without impairing catalysis, while the threonine-to-alanine change at codon 42, but not other substitutions of that residue, promotes increased phosphopeptide binding affinity. The recognition specificity of the C-SH2 domain bearing the E139D substitution differed substantially from its wild type counterpart acquiring binding properties similar to those observed for the N-SH2 domain, revealing a novel mechanism of SHP2's functional dysregulation. Finally, while functional selection does not seem to occur for the substitutions at codons 279 and 468, we point to deamination of the methylated

cytosine at nucleotide 1403 as the driving factor leading to the high prevalence of the T468M change in LS.

#### P01.189

#### **Diagnostic tests for costello syndrome and cardio-facio-cutaneous syndrome - two years service experience**

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Costello and Cardio-Facio-Cutaneous (CFC) syndromes are autosomal dominant multiple congenital abnormalities with phenotypic overlaps to Noonan syndrome. The main difference with respect to their management is an increased tumour risk in Costello syndrome (approximately 17%).

Costello syndrome is associated with mutations in *HRAS* (82-92%), the majority of the mutations identified to date are in codons 12 and 13. No other disease causing genes have been identified. All five coding exons of *HRAS* are screened by bi-directional fluorescent sequencing.

So far four genes have been identified in CFC syndrome; *BRAF* (37-78% patients), *KRAS*, *MEK1* and *MEK2*. We use bi-directional fluorescent sequencing to screen *BRAF* (exons 6, 11, 12, 13, 14, 15 and 16), *KRAS* (all five coding exons), *MEK1* (exons 2 and 3) and *MEK2* (exons 2 and 3).

We have been offering a diagnostic service for both Costello syndrome and CFC syndrome for 2 years, since June 2006. So far 125 patients with a likely diagnosis of either Costello or CFC syndrome have been referred to us. We detected 26 pathogenic mutations in 101 patients tested for *HRAS* mutations. In the cohort of 71 patients tested for CFC syndrome we detected 19 pathogenic mutations (*BRAF*=14, *KRAS*=2, *MEK1*=3) and a further seven that are likely to be pathogenic (*BRAF*=4, *KRAS*=2, *MEK2*=1). We tested both parents of 14 patients with a known pathogenic mutation and 2 patients with a likely pathogenic mutation, in all cases the mutation was shown to be *de novo*.

#### P01.190

#### **Complex Approach to Diagnostics and Data Analysis for Neurofibromatosis type 1**

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The Neurofibromatosis type 1 (NF1) is one of the most common single gene diseases (the expected incidence is 1:3000).

There was implemented a database of 300 records of patients suffering from NF1 in the Institute of Biology and Medical Genetics 2<sup>nd</sup> Medical School, Charles University in Prague.

The complex investigation methodology in the patients with a NF1 has been verified. The DNA bank for these patients was created containing 150 DNA samples. We have implemented reliable molecular genetic diagnostics of the NF1 gene using MLPA and DHPLC methods. We have investigated 39 families and detected causal mutation of the NF1 gene in 26 patients, from which 13 mutations were not previously detected. Last year, we have implemented high resolution melt analysis (HRM) as an effective tool for the mutation scanning of the NF1 gene with sensitivity comparable with the DHPLC method.

The clinical, neurological, neurophysiological, and biochemical data of patients have been analyzed in detail by support of artificial intelligence methods.

The patient records are stored in the form of text files. Their content has to be converted into a database format to analyze them by available machine learning techniques. The pilot data analysis will be summarized.

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#### P01.191

#### **Neurofibromatosis type 1 in adulthood: clinical analysis of a 80 patients cohort**

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Neurofibromatosis type 1 (NF1), a genetic disease, with an estimate incidence of 1/3000. It is a neurocutaneous disorder characterized by multisystemic involvement. NF1 has a wide clinical variability even between family members, with the possibility of mild forms and age specific complications.

Although childhood clinical spectrum and possible complications are well known, few data are available on adult patients, concerning the evolution of the disease, the applicability of a follow up program, the indications for genetic testing, the social and health needs typical of this age.

We describe a clinical cohort of 80 adult NF1 patients, referred to Clinical Genetics Unit at Ospedale Maggiore Policlinico Mangiagalli e Regina Elena. The cohort is composed by 46 females and 34 males patients, age range: 19-69 years. NF1 was inherited from an affected parent in 24 cases (30%). The diagnosis was segmental NF1 in 5 cases, while the other 75 patients have a classical form of the disease.

Clinical features and detected complications of this cohort are compared with the available literature data. Specific attention will be given to the typical and specific needs of adulthood, related to the reproductive life: demand for genetic counselling, recurrence risk, procreative option discussion, prenatal diagnosis request, pregnancy follow up in affected women.

#### P01.192

#### Prenatal diagnosis of neurofibromatosis type 1: Importance of genetic counseling

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with an incidence of about 1 in 3500 live births. Because of the large size and complexity of the NF1 gene, the variety of mutations and the need to identify the specific mutation in each family, indirect diagnosis using linked markers has an important part in genetic counseling.

In this study, thirty-seven families have been characterized and two prenatal diagnoses have been performed by using indirect analyses. Two family were informative for NF1 markers. One of fetuses was under NF1 risk. Now, she is 15 month old, has NF1 features.

The variability of the phenotypic expression of the NF1 gene makes reproductive decisions in NF1 families very difficult. Even mutation detection can not help for decision because molecular diagnosis can not predict the severity of the disease. Therefore good genetic counseling is very important for the couples with the NF1 risk.

#### P01.193

#### Detection of a de novo germline deletion and somatic NF1 mutation in a 2 year-old boy with JMML and multiple café au lait spots

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We describe the history of a child from healthy unrelated parents diagnosed having juvenile myelomonocytic leukemia (JMML) at the age

of 10 months. Since 10% to 14% of patients with JMML have NF1; the present patient had multiple café-au-lait spots allowing to consider for Neurofibromatosis Type I (1,2). The standard karyotype on lymphocytes was 46,XY normal male. Bone marrow karyotype showed monosomy 7. By cDNA sequencing a frameshift mutation in *NF1* gene was characterized, c.2033dupC. With MLPA, total *NF1* gene deletion was observed. These mutations were confirmed on a second independent sample. On fibroblasts, we confirmed the total gene deletion providing evidence for a germline mutation while the c.2033dupC was absent (most likely representing a second hit mutation). Investigation on bone marrow is planned. This is the first report on a *NF1* microdeletion patient with JMML. The finding of a second hit point mutation in a patient with *NF1* microdeletion represents another example of that the type of germline mutation influences the type of second hit in the tumors (3). Loss of heterozygosity (LOH) in the *NF1* region is not an usual mechanism for somatic *NF1* inactivation from tumors in patients with a microdeletion. It is postulated that LOH of *NF1* microdeletion region in these patients would *de facto* lead to a nullizygous state of the genes and might be lethal.

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#### P01.194

#### Germline and somatic NF1 mutations and copy number alterations of chromosome 17 in sporadic and NF1-associated malignant peripheral nerve sheath tumors

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Malignant peripheral nerve sheath tumor (MPNST) is a rare malignancy occurring with increased frequency in patients with neurofibromatosis type 1 (NF1). In contrast to the well known spectrum of germline *NF1* mutations, the information on somatic mutations in MPNSTs are limited. In this study we evaluated the presence of *NF1* mutations in 47 MPNSTs from patients with (n=25) or without (n=22) NF1; from 7 of the NF1-patients neurofibromas were also analyzed. The mutation status was assessed for two other genes, KRAS and BRAF. Mutation profiles were compared with copy number changes for chromosome 17 and TP53 mutation status. Germline *NF1* mutations were detected in 17 NF1-patients: 6 frameshift, 5 truncating, 3 missense mutations, 2 large deletions, and 1 chromosome 17 imbalance. Somatic *NF1* mutations were found in 17/25 NF1-associated MPNSTs, in 3/7 neurofibromas and in 9/22 sporadic MPNSTs. Large chromosomal imbalances accounted for 13/17 and 7/9 somatic mutations in NF1-associated and sporadic MPNSTs, respectively. In the present cohort, 20 NF1-associated and 11 sporadic tumors harbored distal 17q gain. Two NF1-associated and 13 sporadic MPNSTs did not show any *NF1* mutation, arguing for a contribution of other genes. However, a major role of the KRAS or BRAF gene was excluded. Loss of the 17p13 region including the TP53 gene appeared to be implicated both in NF1-associated and sporadic MPNSTs. The present results suggest that somatic and germline *NF1* mutations involved in MPNST development develop by two distinct mechanisms, the former being the same in sporadic and NF1-associated cases.

#### P01.195

#### Early-onset breast cancer in Neurofibromatosis type I

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Neurofibromatosis type 1 (NF1), with an incidence of approximately 1/3500, is one of the most common hereditary disorders. Breast cancer, usually due to somatic rather than inherited gene mutations, is one of the most common malignancies; the lifetime risk for breast cancer in

women ranges by population from 8-12%.

Individuals with NF1 have a well-documented increase in the relative risk of several rare malignancies, including malignant nerve sheath tumors, childhood myeloid malignancies, neurogenic sarcomas, malignant CNS tumors, rhabdomyosarcomas, pheochromocytomas and carcinoid tumors. There has not, however, been a well-documented increase in the incidence of common adult-onset malignancies such as colon, breast and prostate, in individuals with NF1.

Over the past six months in our familial cancer clinic we have encountered two women with a classical presentation of NF1 who developed pre-menopausal invasive ductal carcinoma. A literature and database review initially revealed only anecdotal case reports of breast cancer in NF1, but a recent publication (Sharif et al., J MED GENET 44:481-484, 2007) concluded that women with NF1 under age 50 have an approximately 5-fold increased risk of breast cancer. We next proceeded to a more complete review of publications dealing with mortality and morbidity in NF1 and now agree with the above authors that women with NF1 should be offered earlier mammographic screening because of a moderately-increased risk of pre-menopausal breast cancer. Although a genotype-phenotype correlation might be suspected, such correlations have been difficult in NF1 in general and have not been identified in relation to breast cancer.

#### P01.196

##### Neurofibroma by numbers: mutational mechanisms, models and modifier genes

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The number of dermal neurofibromas (dNFs) in Neurofibromatosis type 1 patients is highly variable. It has been shown that there is an important genetic component in the variation of neurofibroma number that seems to be consistent with a polygenic effect. Neurofibroma initiates due to the double inactivation of the *NF1* gene. Most (if not all) somatic *NF1* inactivations in dNFs are due to mutation, thus genes important for DNA repair mechanisms are good candidates as modifier genes of neurofibroma number variability. We have chosen to study genes responsible for mitotic recombination as candidate modifiers, since mitotic recombination is an important mechanism leading to LOH that accounts for the 20-30% of somatic *NF1* inactivations in dNFs. We are employing different strategies, either directly in humans or using *Saccharomyces cerevisiae* as a model, for identifying candidate genes that will be used in association studies in a well-characterized cohort of NF1 patients.

#### P01.197

##### Mosaic neurofibromatosis type 1 (NF1) in Finland

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**Background** Neurofibromatoses are hereditary diseases that belong to the group of phakomatoses. NF1 has distinctive cutaneous features and NF2 has mainly intracranial lesions. In the segmental or mosaic type (NF5) the disease features of NF1 are distributed regionally on the body. There is no previous data on mosaic NF1 in Finland.

**Methods** Patients' disease features were collected from hospital files. A large literature search was made and the cases were compared with the Finnish patients. The objective was to study the incidence of mosaic NF1 in Finland and the disease features in Finnish patients.

**Results** Classification of patients unequivocally according to existing criteria for mosaic NF1 proved difficult. The incidence of mosaic NF1 in Finland (0,0005%) was lower than that found in other countries (0,0014%-0,002%), but in areas where the material was the most comprehensive (Turku and Oulu University Hospital districts) the incidences (0,0013% and 0,0011% respectively) were very similar to the other studies. The most common disease feature was the neurofibroma, which was found in 81% of the Finnish patients and in 76% of the cases in the literature. The frequency of café-au-lait-spots was respectively 39% and 35% and that of freckles 26% and 38%. Lisch nodules, mainly unilateral, presented in 15% of the Finnish patients

and in 8% of the literature cases.

**Conclusions** There is a need for more unified criteria for classifying mosaic NF1. The incidence and disease features of mosaic NF1 in Finland are similar to those found in other countries and in the literature.

#### P01.198

##### Analysis of the *NF1* gene in a cohort of 118 unselected NF1 patients using direct cDNA sequencing complemented with MLPA analysis

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Neurofibromatosis type 1 (NF1) [MIM 162200] is a common dominant autosomal disorder, affecting 1 in 3500 individuals. The disease is caused by mutations in the *NF1* gene at 17q11.2, in which has been identified a wide range of molecular abnormalities. In the last years it has made clear the need for a reliable and sensitive genetic test for the *NF1* gene to help resolve diagnostic dilemmas in patients not fulfilling clinical criteria as those defined by The National Institutes of Health (NIH).

We screened for mutations a panel of 118 unrelated patients suspected of having NF1, using direct cDNA sequencing complemented with multiplex ligation-dependent probe amplification (MLPA) analysis. Possible disease causing mutations were identified in 82 (69%) cases. These comprised 74 different sequence alterations, of which 46 were novel. Among the 82 changes identified, we observed 26 (32%) frame-shift mutations, 21 (26%) nonsense mutations, 15 (18%) splice errors and 6 (7%) missense mutations. Furthermore, using MLPA approach we found 14 mutations (17%): 8 total gene deletions, 4 multiexon deletions and 2 multiexon duplications.

Out of the 118 cases referred, there were 84 patients with reliable clinical data, of whom 53 satisfied the NIH diagnostic criteria. Within this better defined cohort of NF1 patients, mutations were identified in 47 individuals (89%). Interestingly, 10 out of 24 (42%) patients who satisfied only one NIH criteria carried a mutation.

Our results show that direct cDNA sequencing together with MLPA analysis provide a sensitive and specific method for the rapid identification of *NF1* mutations.

#### P01.199

##### Genetic diagnosis of Neurofibromatosis 1 in 62 Spanish patients and determination of the mutational spectrum of the *NF1* gene in our population

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Neurofibromatosis 1 (NF1) is a multisystemic disease characterized by the presence of multiple café au lait spots, freckling, neurofibromas and iris Lisch nodules. NF1 is caused by mutations in the *NF1* gene, more than 500 different mutations been identified and many of them are private. A half of mutations are *de novo* and the mutational heterogeneity of *NF1* gene is high: point mutations (27%), splicing mutations (20%), small deletion or insertion (47%) and large deletions or rearrangements (8%). The aims of this work are: i) to achieve a genetic diagnosis in patients with clinical criteria of NF1, ii) to evaluate the RNA sequencing of the *NF1* gene as a diagnostic methodology for Neurofibromatosis 1, iii) to determinate the mutational spectrum of the *NF1* gene in our series. Here we report our three years results and experience with genetic diagnosis of NF1. We have achieved a genetic diagnosis in 75 patients and 42 mutations not reported previously have been identified. RNA sequencing of the *NF1* gene is the methodology gold standard for the genetic diagnosis of Neurofibromatosis 1, because it allows to identify point mutations, splicing mutations and duplications or deletions, including these affecting to one or a only a few exons. The mutational spectrum of the *NF1* gene obtained in our population provides an interesting epidemiologic and pathogenic information. A high expertise and experience must be required for the interpretation of nucleotide changes identified in the sequencing of genes with a high rate of *de novo* mutations

**P01.200****UMD-DMD France: A national knowledgebase of molecular defects in the dystrophin gene**

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UMD-DMD France is a national locus-specific database (LSDB) dedicated to dystrophinopathies. It has been developed through a multi-center academic effort to provide an up-to-date resource of curated information covering all identified and fully validated mutations in patients with dystrophinopathies in France. Whenever necessary, mutations have been reevaluated at the light of the currently available techniques. The database includes 2270 entries corresponding to 2070 independent mutational events identified in either male patients (2034) or symptomatic female carriers (36). These mutations consist in 1420 deletions, 261 duplications, and 449 small rearrangements of which 39.1% are nonsense. Most of them are unpublished. Experts in the DMD gene and related diseases are responsible for data quality and accuracy. In addition to gather mutations, the UMD-DMD France includes available data on dystrophin and RNA analysis, phenotypic groups, and transmission. The database aims to include extensive description of phenotypes associated with the reported mutations to better delineate the clinical spectrum of dystrophinopathies and to allow genotype/phenotype correlations. In addition to the existing routines in the UMD software, new tools have been specifically developed to facilitate large-scale mutation analyses of the DMD gene. UMD-DMD is a searchable anonymous database which will benefit to all the scientific community interested in dystrophinopathies including geneticists, clinicians, and researchers involved in the design of therapeutic strategies. It will prove useful also to implement forthcoming global registries of patients for clinical trials as ultimate goal within the European network of excellence for treatment of neuromuscular disorders (TREAT-NMD).

**P01.201****Analysis of duplications within the DMD gene**

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The DMD gene is one of the largest human genes identified, spanning more than 2000 kb of genomic DNA encoding a 14-kb transcript. It is known that 60 to 65% of the mutations causing DMD/BMD are large deletions in the dystrophin gene. The deletions are nonrandomly distributed and occur primarily in the center (80%) and less frequently near the 5' end (20%) of the gene. The large size of the gene may account for part of the high deletion rate. However because of the nonrandom distribution of the deletions, in addition to target size, other factors must be involved like variation in chromosomal stability.

Although partial gene duplications in the DMD gene were reported to be relatively frequent (5% - 10%), only recently - with the development of MLPA - detection of duplications in male patients and deletions and duplication in female carriers became feasible in a routine setting and first data concerning frequency and distribution of duplications within the DMD gene became available.

These data show that unlike the deletion distribution, the majority of the duplications (80%) are found at the 5' end of the gene and only 20% in the central region suggesting that deletions and duplication originate by different mechanism.

To investigate the mechanism causing duplications in more detail and to compare this mechanism with mechanism causing deletions in the same region of the gene we precisely mapped duplication breakpoints

and sequenced six of them. First data of these comparisons will be presented.

**P01.202****Congenital Muscular Dystrophy with heterozygous mutations in LAMA2 and FKRP**

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A child presenting with hypotonia and muscle weakness since infancy, ambulation until twelve years of age, seizures and normal cognitive function is described. CK was elevated (650-1420 IU/L). Brain MRI showed a few abnormal T2 white matter changes. Muscle biopsy showed dystrophic features and skin biopsy demonstrated absence of merosin immunostaining (300 kd antibodies, Novocastra Laboratories). Molecular genetic studies detected heterozygote mutations in both the LAMA2 (Val1754X) and FKRP (A114G ) genes, which were traced to the father and to the mother respectively. Two subsequent sibling fetuses carried the LAMA2 mutation. In one of them the FKRP mutation was also detected in one allele and in the other the FKRP gene was normal at both alleles. Even though the genotype of the two aborted fetuses and the patient were identical at the LAMA2 locus, the fetuses had normal merosin staining in chorionic villus sampling, whereas the patient was merosin-negative. Our findings suggest an additional potential interactive mechanism to LAMA2, possibly of the FKRP protein which is essential for preserving the sarcolemma integrity. These findings suggest that merosin-deficiency could be the result of di-generic inheritance.

**P01.203****Genetic testing and counseling for Facioscapulohumeral Muscular Dystrophy (FSHD) in Israel, 2000-6**

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In the last few years our laboratory at Wolfson Medical Center has been the only lab in Israel to perform genetic testing for FSHD, a common, dominantly inherited, late onset, progressive, but relatively mild type of muscular dystrophy, with considerable clinical variability even within families. No treatment or prevention of symptoms are available, prenatal diagnosis is complicated and pregnancy termination of affected fetuses - ethically controversial. FSHD genetic testing is important for prognosis and genetic counseling, since clinical overlap exists between FSHD and other LGMDs.

The gene causing FSHD has not been identified, but molecular diagnosis can be made by analyzing D4Z4 repeat length on chromosome 4q35. Results can support or rule out the clinical diagnosis of FSHD, but there are also "gray zone", non-conclusive results.

66 individuals were tested during the years 2000-6, including 7 asymptomatic individuals.

We present our results and conclusions and recommendations regarding the percentage of conclusive results obtained after FSHD genetic testing, cognitive involvement frequency and anticipation in our patient sample. Additionally, based on our results, recommendations for genetic counseling of individuals and families referred for FSHD testing are given, including addressing the issue of presymptomatic testing.

**P01.204****A novel mutation in FKRP gene in Italian patient with LGMD**

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Limb-girdle muscular dystrophy type 2I (LGMD2I) is an autosomal recessive muscular dystrophy caused by mutations in the gene coding for fukutin-related protein (FKRP). LGMD2I is clinically heterogeneous, and it is allelic to MDC1C (congenital muscular dystrophy type1C) with onset in the neonatal period. The function of FKRP in skeletal muscle is largely unknown; however, indirect evidence suggests that FKRP may be a putative glycosyltransferase involved in  $\alpha$ -dystroglycan ( $\alpha$ -DG) processing. Muscle biopsies of patients affected by either LG-

MD2I or MDC1C show a variable reduction of α-DG glycosylation. We report a 70 year-old man he was referred for muscle weakness of the lower limbs starting at age 20 and mild but progressive hypotrophy of girdle. After informed consent, mutations screening was performed on genomic DNA in regard to of CAV3 and CAPN3 genes. The patient found negative screening of these genes, has been investigated for mutations of FKRP gene. The mutation analysis showed a missense change (Ser115Leu), in heterozygous state, never described. To confirm that the alteration found was mutation and not polymorphism, a screening was carried out with 100 control Caucasian chromosomes. In conclusion this mutation of the FKRP gene could even be responsible for mild phenotype of the patient.

#### P01.205

##### Mutation analysis of the CAPN3 gene in Italian patients with suspected LGMD type 2

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Autosomal recessive limb girdle muscular dystrophies (LGMD type 2) are a clinically and genetically heterogeneous group of disorders, characterized by progressive involvement of proximal limb girdle muscles. LGMD2A (MIM# 253600), whose locus has been mapped to chromosome 15q15.1, is considered to be the most frequent form of recessive LGMD. LGMD2A is caused by single or small nucleotide changes widespread along a 40-kb gene, named CAPN3 (MIM# 114240), which encodes for the calpain-3 protein. Aims of this study were to assess the frequency of mutations in 30 Calabrian patients suspected diagnosis LGMD and identify possible genotype-phenotype correlation as prognostic factors. At the screening of gene CAPN3 have been identified already described mutation (R748X) in exon 21 and a new polymorphism (CTCT) in intron 14, while all other samples had already described polymorphisms. The subject bearer of R748X mutation in heterozygosity had a mild phenotype, in fact related onset of disease at 48 years and the muscle biopsy showed a slight myopathy associated with a pain and weakness in the lower limbs. In particular, this mutation was associated with another exchange in exon 5 Ala236Thr described in the literature as polymorphism. However, the combination of a polymorphism in one copy of the CAPN3 gene with an LGMD2A mutation in the other copy could even be responsible for very mild phenotype with low penetrance. Patients with LGMD2 phenotype in the absence of mutations in CAPN3 should be investigated for the genes responsible for other forms of LGMD2.

#### P01.206

##### Myopathy - an early finding in several rare genetic syndromes

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Introduction. Myopathies are part of the larger group of neuromuscular disorders. The main clinical manifestation is muscular weakness. Myopathy is associated with over 150 hereditary entities and may be the first clinical manifestation in several rare genetic syndromes. Objective. To present a case series of rare monogenic syndromes that were initially diagnosed based on the finding of myopathic features. Results. We are presenting a case of Schwartz-Jumpel syndrome with familial distribution (consanguineous parents with three affected daughters), a case of Dubowitz syndrome with familial distribution (consanguineous parents with two affected sons) and a case of Camurati-Engelmann syndrome. In all cases, the first manifestations of disease that prompted medical care were myopathic in nature. Conclusion. Muscle weakness in children, if no clear cause is found, should prompt further investigation to rule out rare genetic disorders.

#### P01.207

##### Prenatal diagnosis of Duchenne/Becker muscular dystrophy in Serbia-twelve years experience

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In Serbia, prenatal testing for Duchenne/Becker muscular dystrophy (D/BMD) is an option for women at risk of having an affected son,

since 1996.

During period 1996-2007 in total 54 prenatal tests were performed in 43 families. From these, 34 pregnancies were male pregnancies at risk of D/BMD and 8 of them (23.5%) showed an increased risk.

DNA extraction was done from chorionic villuses samples in 41 (76%) cases, amniotic fluid cells in 11 (20.3%) cases and in 2 cases (3.7%) from fetal blood samples. Prenatal diagnosis was based on direct detection of the mutation in 51 cases. In 49 of them, we used standard multiplex PCR for simultaneous amplification of 18 DMD exons. In two cases MLPA was applied for determining carrier status of mothers and the detection of the duplications in DMD gene of fetuses. In 3 cases (5.55%), with unknown mutation in proband, prenatal diagnosis was done by indirect DNA analysis. Maternal contamination has been excluded in all cases using polymorphic CA markers.

Since no clinically applicable and effective therapy for DMD patients has yet been developed, a molecular diagnosis should be proposed to the families in order to detect the carrier women and to suggest an antenatal diagnosis.

#### P01.208

##### Genetic testing, carrier detection and prenatal diagnosis of 300 Egyptian families with Duchenne muscular dystrophy: a 10 years experience

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Duchenne Muscular Dystrophy (DMD) is the most common lethal X-linked recessive muscle disorder that is caused by mutations in the dystrophin gene. Due to lack of efficient rehabilitation and treatment, prenatal diagnosis and counseling of families with DMD are of great importance. Here we report a 10 years Egyptian experience on the molecular diagnosis and genetic counseling of 300 families with DMD at the National Research Centre, Cairo.

300 probands with DMD were screened for dystrophin gene deletion mutations by multiplex PCR. Four CA dinucleotide repeats and three restriction fragment length polymorphisms (RFLPs) were used to detect the carrier status in 35 families. Prenatal molecular diagnosis was pursued for 32 pregnant mothers at risk of having a child with DMD by multiplex PCR when there is a deletion mutation in the affected sib or by intragenic markers in families with no deletion mutations.

55% of probands had deletion mutations. 60% of detected deletions involved multiple exons spanning the major and/or minor hotspots of the gene while 40% involved single exon deletions. The combined use of CA repeats and RFLPs detected the carrier status in all families identifying 20 female carriers. Molecular diagnosis of the fetal DNA showed that 15 fetuses inherited the same deletion mutations present in the index cases. A high acceptability of seeking prenatal diagnosis and a change in attitude towards the decision of abortion was noticed.

Molecular diagnosis, carrier detection and prenatal diagnosis are effective tools for definitive diagnosis and genetic counseling in families affected with DMD.

#### P01.209

##### Prenatal diagnosis in a DMD family at risk

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A 23-year-old pregnant woman is the sister of a patient who is affected by Duchenne muscular dystrophy (DMD). Her brother, an 18-year-old male, was detected to have a novel mutation in exon 6 of the DMD gene (c.587delAT). She asked for prenatal diagnosis to detect whether her unborn child has DMD or not. A prenatal diagnosis by chorionic villi sampling (CVS) was performed at 10 weeks of pregnancy. Analysis consisted of PCR amplification followed by direct sequencing of the entire coding region of the DMD gene. The fetus showed a normal male karyotype (46,XY) and no abnormality of the dystrophin gene. In this case, prenatal diagnosis by CVS was able to exclude DMD in the unborn child.

**P01.210****The high frequency of new mutations in dystrophin gene in the group of Polish patients affected with Duchenne?Becker muscular dystrophy**

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The frequency of Duchenne/Becker muscular dystrophy (DMD/BMD) among the newborn males is 1: 3500. The constant of DMD/BMD incidence in male population is the evidence of the stable frequency of new mutations. Although men affected with DMD/BMD usually are not able to reproduce incidence of the disease remains the same. It is assumed that 1/3 of single DMD/BMD male are the cases of new mutations.

The study of carriership was carried out in a group of 249 mothers with one son affected with DMD/BMD in whom deletions in dystrophin gene were found. The studies of microsatellite sequences in the mothers revealed the presence of two different alleles in 138 females, therefore their carriership could be excluded. In 38 cases, in which mothers or daughters (sisters of affected males) were tested, the presence of the deletion was observed. The homozygosity was detected among 73 females. Some of them may be instances of hemizygosity. The exclusion of carriership in 55% of tested females is statistically different from the expected incidence of 33%.

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**P01.211****Two novel mutations in a Russian family with X-linked Emery-Dreifuss muscular dystrophy**

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Emery-Dreifuss muscular dystrophy (EDMD) is a neuromuscular disease characterised by early contractures of Achilles-heel, elbows and spine, slow progression and symmetric weakness prominent in humero-peroneal muscle, cardiac conduction abnormality and/or cardiomyopathy and myopathic features. Two main modes of inheritance exist: X-linked and autosomal dominant. The frequency of the X-linked form is at 1:100 000. *EMD* gene mutations, which encodes a nuclear membrane protein named emerin, are causes of the X-linked form of EDMD. *EMD* gene has been mapped to the region Xq28. Mutation analysis in *EMD* gene was performed by direct automatic sequence on Genetic Analyzer 3130 of all exons and exon-intron splices. We describe two novel mutations in the *EMD* gene in two Russian families. Mutations change nucleotide sequence of the sixth exon in the *EMD* gene. Family 1 had a c.664C>T (p.Gln222Stop) which causes a premature stop-codon at position 222. In second family we found the mutation c.449+1delG which causes a disappearance donor splicing-site and subsequent - a premature stop-codon at position 221.

**P01.212****Genotype-phenotype correlates in autosomal recessive Myotonia Congenita**

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Myotonia Congenita (MC) is a chloride channel disorder due to mutations in the CLCN1 gene, inherited in autosomal dominant or recessive fashion. Beside myotonia of variable severity, transitory weakness (TW) can be detected, especially in recessive cases. Neurophysiologically, TW corresponds to a transitory depression (TD) of the compound muscle action potential during repetitive nerve stimulation (RNS). We sought to correlate specific CLCN1 mutations with both TW and RNS-induced TD in 28 patients with recessive MC, by adopting a low frequency RNS protocol (3Hz for 500 stimuli). The 3Hz RNS induced a significant TD in 19 patients, all of whom experienced variable episodes of TW. Several mutations appeared to correlate with specific TD patterns. In particular, the homozygous exon 9 deletion, C481X

and IVS1+3A>T mutations (5 cases), as well as the compound heterozygous mutations G482R and T550R (1 case), always resulted in marked TD. Conversely, F167L seems to associate with mild or no TD, since 8 of 10 patients carrying this mutation (in compound heterozygosity with other mutations) had clinical absence of TW and a negative RNS test. Finally, the 3 patients heterozygous for the A531V change (compound with either R377X or R894X) presented a peculiar pattern of fluctuating TD. This study shows that the 3Hz RNS protocol, well tolerated by patients, is able to identify distinct neurophysiological TD patterns correlating with specific CLCN1 mutations. In turn, this may help understand the molecular basis underlying the phenotypic variability of MC, and may represent a step towards rational therapeutic strategies.

**P01.213****DNA-diagnostics of myotonic dystrophy type 1 in Belarus**

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant, multisystemic disease, with an estimated incidence of 1 in 8000. DM is caused by expansion of CTG trinucleotide repeats located in the 3' untranslated region of the myotonin protein kinase (DMPK) gene. The range of expansion varies widely from 50 to several thousand repeats. First, we analysed the distribution of CTG-repeats in a cohort of normal individuals from the Belarusian population, and assessed the heterozygosity and number of alleles. PCR-products were analysed by the automated capillary electrophoresis on the ABI Prism 310. We found 15 different allelic variants from 5 up to 28 CTG-repeats. The most common alleles had 5 repeats (38%). The heterozygosity of the CTG polymorphism of the Belarusian population was established as 78%. DNA-diagnostics of DM1 was done in 75 patients from 32 families showing different symptoms related to the disease. We found small expansions with 70 and 86 CTG repeats in two affected men from two different families. PCR analysis also shows the high risk of mutation of the DMPK gene in 10 patients from 3 families, further confirmed by the Southern blot. So our PCR-based protocol allows amplification of normal-sized alleles and small expansions. For the large expansion Southern blot analysis is still the method of choice.

**P01.214****Molecular studies on two myotonic dystrophies (DM1 and DM2) in Polish patients group**

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Myotonic dystrophies type 1 and type 2 (DM1 and DM2) are autosomal dominant disorders and share similar symptoms comprising myotonia, muscular dystrophy, and multisystem involvement (cataracts, diabetes and hypogonadism), but overall clinical picture of these two conditions is not identical.

Molecular defect causing DM1 and DM2 is known as dynamic mutation in DMPK and ZNF7 genes respectively. Expansions up to 4000 CTG repeats result in DM1 whereas a CCTG tetramers up to 11000 in patients with DM2 are observed.

Molecular testing is the only reliable diagnostic definition in DM1 and DM2. Prior to its introduction we have performed the analysis of normal alleles distribution in Polish control group. For DM1 locus the range is 5-31 repeats and for DM2 the range of repeat motif varied from 123 to 159 bp.

For 5 years 372 individuals were tested for DM1 and/or DM2 by routine PCR, then all homozygous cases were analyzed by TP-PCR. Among them we have identified 76 pedigrees with 144 DM1 and 34 pedigrees with 35 DM2 mutation carriers. Within the group of DM1 patients we identified one family with a case of premutation which resulted in full mutation in the following generation. Moreover, we identified a patient with a coexistence of full mutation and premutation in DMPK gene. Among DM2 group we came across a family with two patients having extended alleles - 189 bp and 197 bp i.e. clearly above the normal range but below the considered as pathogenic range in DM2 (normal range 104-176 bp).

**P01.215****The CTG repeat expansion size correlates with the splicing defects observed in muscles from myotonic dystrophy type 1 patients**

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Myotonic dystrophy type 1 (DM1; MIM#160900) is caused by an unstable (CTG)n repetition located in the 3'UTR of the DMPK gene. Untranslated expanded DMPK transcripts are retained in ribonuclear foci and sequester CUG-binding proteins essential for the maturation of pre-mRNAs. In this study, we investigated the effects of CTG expansion length on three molecular parameters associated with the DM1 muscle pathology: 1) the expression level of the DMPK gene; 2) the degree of splicing misregulation and 3) the number of ribonuclear foci. To this purpose, we selected 6 muscle biopsies from DM1 patients with an expansion below 500 repetitions, 6 muscle samples from DM1 patients carrying a mutation above 1000 CTGs and 6 controls muscle samples. Splicing analysis of the IR, MBNL1, c-TNT and CLCN1 genes demonstrated that the level of aberrant splicing isoforms is strikingly different between the two groups of DM1 muscle samples. In addition, a significant correlation was observed in the extent of abnormal splicing and the CTG repeat length for all the genes studied. RNA-FISH analysis reveals that the number of ribonuclear foci accumulating in DM1 muscle sections increases in patients with a higher (CTG)n number. On the contrary, we did not find any relationships between the expression level of the DMPK gene transcript and average expansion sizes. These data indicate that the CTG repeat length plays a key role in the extent of splicing misregulation and foci formation, thus providing a useful link between the genotype and the molecular cellular phenotype in DM1.

**P01.216****The results of a systematic screening for new mutations in NAIP gene**

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**Background:** The NAIP gene is located in an inverted duplicated region (5q13), enclosed to some repetitive elements which predispose to chromosomal rearrangements. However, a small number of NAIP mutations/polymorphisms were reported in this region which is considered a hot-spot mutational. The most common NAIP mutation is a deletion involving exon 5, which has been reported to be associated with spinal muscular atrophy (SMA) complications.

**Aim:** The aim of this study was to screen the NAIP gene for new mutations/polymorphisms.

**Material and methods:** We selected for this study SMA patients (n=63), dialyzed patients (n=200) and healthy clinical subjects (control group, n=370) after informed consent obtaining. The DNA samples for all subjects were screened for presence of NAIP exon 5 by PCR. We also used specific molecular methods to test the presence of additional mutations in this region.

**Results and discussion:** The homozygous absence of NAIP exon 5 was observed in 20,6% SMA patients and in 1% of dialyzed and control subjects. The restriction pattern (DraI) and the indirect methods have been shown that a sample from dialyzed lot has an abnormal electrophoretic and melting comportment. The sequencing analyses have confirmed a heterozygous state for a G/T substitution (Arginine/Serine), which creates a new restriction site for DraI endonuclease.

The etiology of renal failure could not be established.

**Conclusion:** In this study we have identified a new coding G/T SNP in NAIP gene and the highest frequency of homozygous NAIP exon 5 deletion in SMA patients (20,6%).

**P01.217****Soluble expanded PABPN1 exacerbates cell death in Oculopharyngeal Muscular Dystrophy**

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant late-onset myopathy characterized by progressive eyelid drooping, swallowing difficulty and proximal limb weakness. OPMD results from the expansion of a polyalanine repeat (GCG)8-13 in exon 1 of the *poly(A)binding protein nuclear1* (PABPN1) gene. Nuclear aggregates consisting of tubular filaments within muscle fibers of OPMD patients are the pathological hallmark of the disease. These aggregates contain expanded PABPN1 (expPABPN1), poly(A)RNA as well as components of the ubiquitin-proteasome degradation pathway and molecular chaperones. Whether nuclear aggregates are pathogenic or simply the consequence of a molecular defense mechanism remains controversial in OPMD and in the field of neurodegenerative disorders. To evaluate the contribution of nuclear aggregates to cellular toxicity, we first targeted molecular mechanisms known to interfere with expPABPN1 aggregation. Our cellular model shows that increasing the availability of nuclear soluble expPABPN1 significantly exacerbates cell death. We also used time lapse imaging to follow the evolution of cells overexpressing expPABPN1 without interfering with any cellular pathway. Cells with nuclear aggregates show a significantly prolonged lifespan compared to cells harbouring a diffusely distributed soluble expPABPN1. This is the first report indicating the beneficial effect of nuclear aggregation in OPMD. The formation of nuclear aggregates may reflect an active process by which cells sequester and inactivate the soluble toxic form of expPABPN1. The structural change in expPABPN1, induced by the pathogenic alanine expansion, may lead to a gain of aberrant protein interactions, or alternatively prevent normally occurring interactions to take place.

**P01.218****Spinal muscular atrophy in University Hospital Brno, Czech Republic- Genetic counselling, DNA analysis, Prenatal analysis**

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Spinal muscular atrophy (SMA) is the second most frequent lethal autosomal recessive disease in Europeans with the incidence of 1/6000 -10 000 and a carrier frequency of 1/40-50. SMA is in approximately 96% of cases caused by homozygous deletion of the SMN1 gene. 4% of SMA patients have a combination of the deletion or conversion in one allele and an intragenic mutation on the second one. The SMA-determining gene (survival motor neuron - SMN), is present on 5q13 in two copies, a telomeric SMN1 gene and a centromeric SMN2 gene, which are highly homologous and contain only five base-pair differences. However, increased SMN2 gene copy number, which can occur as the result of gene conversion events, is associated with a milder SMA phenotype.

Since 1999, we have performed the DNA analysis of SMA, the carrier detection from 2003 and from 2005 we analysed the copy number of SMN2 gene.

Until now, we examined more than 300 SMA patients, only one SMN1 copy was detected in 17 of them, we identified 6 point mutations.

Currently, we perform the genetic counselling in families with SMA occurrence, we offer DNA analysis of the SMN1 gene in the proband and the detection of SMA carriers. We offer the DNA analysis of SMN1 gene for partners of SMA carriers. In families with high risk of SMA in the child we offer prenatal DNA diagnostics from cultivated amnial cells.

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**P01.219****Accuracy of marker analysis, quantitative real time PCR and Multiple Ligation-dependent Probe Amplification to determine SMN2 copy number in patients with spinal muscular atrophy**

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Spinal muscular atrophy (SMA) is an autosomal recessive disease characterised by degeneration of motor neurons of the spinal cord and is caused by mutations in the SMN1 gene. The SMN2 gene is the highly homologous SMN1 copy that is present in all patients. There is

evidence that the number of SMN2 copies acts as a phenotypic modifier. Acute type I patients usually have one-two copies and chronic type II and III patients usually have three-four copies. Traditionally, the linkage analysis with C212 and C272(ag1-CA) markers was helpful in estimating the SMN2 copies. Quantitative real time analysis (Q-RT) and recently multiple ligation-dependent probe amplification (MLPA) have been incorporated into laboratory diagnosis to establish the SMN2 copies with greater accuracy.

We compared the SMN2 copy number of 22 unrelated Spanish SMA patients with SMN1 absence, correlating the three aforementioned approaches. Using marker analysis, we determined the maximum number of alleles by C212 or C272(ag1-CA), estimating the number of SMN2 copies. The marker results of the respective parents were used to confirm the copy number. In 4 cases, marker analysis predicted two SMN2 copies and Q-RT (LightCycler) and MLPA revealed three SMN2 copies. This discordance may be the result of an uninformative marker. In 2 other cases, marker results predicted at least three SMN2 copies although Q-RT showed two copies and MLPA results were compatible with three copies. This discordance may reflects the lack of probe hybridisation in some samples and highlights the importance of matching results of the three approaches. FIS05-2416/CIBERER/Proyecto GENOME.

#### P01.220

#### DNA diagnostic of Duchenne-Becker muscular dystrophy in Belarus

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Duchenne-Becker muscular dystrophy (D/BMD) is X-linked recessive muscle-wasting disease with incidence 1:3500 and 1:20000 of male newborns respectively. The cause of this disorder is mutations of the gene DMD, located on locus Xp21. The most frequent mutation - gross deletions in «hot spot» regions of 2-19 and 41-53 exons, which detected in 35-75% of cases in different populations.

We have performed molecular-genetic diagnostic in cohort of 52 belarussian D/BMD patients. All of them had typical clinical features and high level of CPK. To detect deletions of Pm/1, 3, 4, 6, 8, 12, 13, 16, 17, 19, 32, 34, 41-45, 47-53 and 60 exons used both multiplex and routine PCR methods. 20 of 52 (38%) patients had deletions of different size and localization. Only 7 patients had one exon deletion. The largest deletion we had found was deletion of 35 exons (region 8-44 exons). In 4 of 20 cases exon 48 was deleted. 35% of mutations localized in proximal part of gene DMD.

Thought, multiplex PCR is rapid and suitable method to detect gross deletion, due to low level of its occurrence in Belarus, there is need in other methods to find both duplication and point mutation within gene DMD.

#### P01.221

#### Molecular genetic analyses of the dystrophin gene in Hungarian Duchenne/Becker muscular dystrophy patients: Comparison of multiplex PCR, Southern blot and MLPA analyses

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Duchenne/Becker muscular dystrophy (DMD/BMD) is a severe X-linked neuromuscular disease caused by mutations in the dystrophin gene. Deletions, rarely duplications and point mutations can occur almost anywhere in the gene, which makes the molecular diagnosis difficult. Here we present a comprehensive study of a large portion of the Hungarian DMD/BMD families using different molecular approaches. Deletions in the hot spots regions were identified by multiplex PCR, whereas rare deletions and duplications were detected by Southern blot analysis and Multiplex Ligation-dependent Probe Amplification (MLPA) technique in the patients. Moreover, the same techniques were used for detecting carrier status in female relatives and in manifesting carriers and efficiencies of the two techniques were compared.

Here we report the genetic results of 121 affected males and 95 female relatives. The DMD/BMD disease was confirmed in 77 males using multiplex PCR. With Southern blot analyses and later on, by MLPA

rare exon deletions were detected in 7 male patients, whereas duplications were observed in 5 cases. Thus, the overall deletion frequency was 69% in the Hungarian DMD/BMD patients. Out of the 95 female samples analysed by Southern blot and MLPA techniques, 41 female relatives proved to be carriers, including two duplication events and three manifesting carriers.

With the help of this reliable new method a large portion of the Hungarian DMD/BMD patients and their female relatives were exactly genotyped and given a precise genetic counselling. Moreover, this opens the perspective for participation in future therapeutic interventions, also for the Hungarian patients.

#### P01.222

#### Three mutations in the *DYSF* gene in a LGMD2B patient

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Mutations in the dysferlin gene (*DYSF*) give rise to different muscular dystrophy phenotypes with autosomal recessive inheritance including Limb Girdle Muscular Dystrophy 2B (LGMD2B), Miyoshi Myopathy (MM) and Distal Anterior Compartment Myopathy (DAT). The *DYSF* gene, which maps to chromosome 2p13, has 55 exons and codifies a protein of about 237 kDa. Given that dysferlin is expressed in peripheral blood monocytes in addition to skeletal muscle, we performed the screening of mutations in the *DYSF* gene by sequencing monocytes cDNA. This strategy improves the isolation of the mRNA as a source that is less invasive than the muscle biopsy.

In parallel, we analysed dysferlin expression by immunohistochemistry in muscle biopsies and by Western blot in peripheral blood monocytes.

We report here an sporadic case of LGMD2B, presenting a reduced staining in immunohistochemistry and Western Blot analyses using anti-dysferlin antibodies.

The mutational screening revealed the presence of three mutations:

- 1) A nonsense mutation located in exon 34: **c.3805 G>T; p.Glu1269X**.
- 2) A missense mutation located in exon 44: **c.4820 T>C ; p.Ile1607Thr**.
- 3) A splice site mutation located in intron 21: **c.2055+1 G>A**, a mutation not described to date.

The independent sequencing of the two alleles will enable us to determine the distribution of the mutations. The distribution of the three mutations could account for the reduced expression of dysferlin in muscle biopsy and monocytes in contrast to the total absence in dysferlinopathy patients.

#### P01.223

#### Genotype and phenotype studies of myotonic dystrophy type 1 (DM1) in Hungarian patients

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Dystrophia myotonica type 1 (DM1) is a diffuse systemic disorder and is inherited as an autosomal dominant trait with a variable penetrance. An unstable expansion of (CTG)n repeats in the 3' untranslated region encoding a member of the protein kinase family in 19q13.3 is the causative mutation for myotonic dystrophy. Healthy individuals harbour 5-37 CTG repeats, whereas in affected individuals repeat expansion varies between 37 and 4000. To examine the correlation between clinical expression and CTG trinucleotide repeat length, Southern blot analyses using probe p5B1.4 were carried out in families clinically diagnosed with myotonic dystrophy. So far, 61 patients and 15 family members from 47 families were analysed and in 34 cases the mutation in DMPK gene was confirmed. The expanded CTG repeats were transmitted maternally as well as paternally. In the maternally transmitted cases the expanded fragment lengths were always larger than in the paternally transmitted ones. Moreover, a clear correlation was established between phenotype severity and the length of the CTG ex-

pansion. Longer expansions resulted in earlier onset of the symptoms. Phenotypes varied between congenital onset, classical forms and mild symptoms even within the same family corresponding to the size of the expansion.

Additionally, we were able to offer prenatal diagnosis in three families where all foetuses inherited the pathogenic DMPK gene. In two foetuses with maternal inheritance the estimated size of the CTG repeat expansion predicted a congenital form, whereas one foetus with paternal inheritance inherited a moderate expansion size. This latter baby has been born recently.

#### P01.224

#### Normal variation of (CTG)n repeat in the Dystrophia Myotonica Protein Kinase gene in Slovak non-Romany and Romany population

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Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder caused by a CTG trinucleotide expansion in the Dystrophia Myotonica Protein Kinase gene (DMPK). This repeat is highly polymorphic in healthy population with alleles in a range of 5 to 37 CTG repeats. The CTG expansion can vary from 50 to several thousand repeats in affected individuals. In many populations healthy alleles show trimodal distribution with 5, 9-17 and 18-35 repeats. A correlation between the frequency of large-sized normal alleles and the prevalence of DM1 in different ethnic groups was shown in several studies. We have analyzed the CTG repeat length in samples of healthy, unrelated individuals from the Slovak Romany and non-Romany population by PCR with fluorescent labelled primers using fragment analysis in genetic analyzer. We have found larger number of different alleles in the sample from non-Romany population than from Romanies, however the (CTG)<sub>n</sub> allele size range was broader in the latter group. In both populations we found trimodal distributions with the majority of chromosomes belonging to the groups (CTG)<sub>5</sub> and (CTG)<sub>9-17</sub>. Our results show lower frequency of (CTG)<sub>5</sub> and (CTG)<sub>18-37</sub> and higher frequency of (CTG)<sub>9-17</sub> alleles in the Romany than in the non-Romany population. Since many linguistic and genetic studies place the origin of Romanies to Indian subcontinent, we decided to compare our results to the available data about (CTG)<sub>n</sub> allele frequencies in European and Indian populations. Our preliminary comparison showed similar distribution pattern among Slovak Romany and Indian population, and among Slovak non-Romany and mixed European population.

#### P01.225

#### Application of western blot for analyzing of Dystrophin in Iranian patients with mild Dystrophinopathy

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Dystrophinopathies (Duchenne muscular dystrophy and Becker muscular dystrophy) are X-linked recessive disorders manifesting with muscle degeneration and weakness. The gene which is defective in Dystrophinopathies is the largest known gene, consisting of almost 0.1% of the human genome (2,500 Kbp). The product of this gene in normal muscle, Dystrophin, is a 427 kDa rod-shaped protein. Dystrophin is an essential part of a large complex that links the actin cytoskeleton with the cell membrane and the extracellular matrix and stabilizes the myofibers during contractions. The value of analyzing Dystrophin on western blots of skeletal muscle for the differential diagnosis of Xp21 muscular dystrophies is now fairly well established especially for mild forms of the diseases (BMD) which immunohistochemistry techniques are not sufficient for the definite diagnosis. Here we describe a sensitive system based on monoclonal antibodies to Dystrophin. System has been set up using GAPDH protein as control that extracted from K562 cells. The specificity of the antibodies was established by dot blot and experiments were undertaken to identify the source of Dystrophin-related protein bands which were detected on blots of normal skeletal muscle. In our study which was the first application of western blot analysis in muscle disorders in Iran, we examined muscle samples taken from clinically suspected to BMD and DMD, first by immunohistochemistry methods and then by western blot analysis. Results show the necessity of blotting techniques in diagnosis panel of mild forms of Dystrophinopathies.

#### P01.226

#### Charcot-Marie-Tooth disease 1B and phenotype-genotyping correlation

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DNA analysis of 96 CMT1-patients revealed 76 cases (79%) of dup17p11.2, (PMP22 gene), point mutation CX32 in 15 cases (16%), and 5 point mutations in the MPZ gene (S63F, R98C, K130R, D134E and not previously described P133S).

In three unrelated families (15 affected subjects) mutation K130R in MPZ was revealed in two families and D134E mutation in one family. All patients has early manifestation of CMT disease before five years old. Late onset of independent walking (18-24 month of birth), unsteady gait, discoordination, distal paresis, muscular atrophies, proprioceptive and superficial hyperesthesia of feet and hands, generalized tendon areflexia, ataxia, foot deformity and nervous swelling were present in all patients with K130R and D134E mutations.

In the patients with R98C and D134E mutations Dejerine-Sottas syndrome with hearing loss was observed. In contrast, the S63F mutation leads to a slowly progressive disease. Independent ambulation was possible until 40-50 years old.

All patients have very low MNCV n. medianus 12,4 +2,4 m/s, n. tibialis - 8,8 +2,6 m/s, distal motor latency 12,5 +5,1 ms; 21,3 +8,7 ms, and very low C - 0,27 +0,18 mV during ten years from two years old. This suggest that abnormalities of nervous fiber myelination may be congenital in origin.

#### P01.227

#### A novel mutation in GDAP1 and a change in MFN2 genes in a family with a severe form of Charcot-Marie-Tooth

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Recessive form of Charcot-Marie-Tooth disease with hoarseness (CMT2K, MIM#607831) is caused by mutations in ganglioside-induced differentiation-associated in protein 1 (GDAP1) (MIM606598), located in chromosome 8 (8q21.1). Dominant axonal forms of CMT (CMT2A2) (MIM#609260) can be caused by mutations in the mitochondrial fusion protein mitofusin 2 (MFN2) (MIM608507), in chromosome 1 (1p36.2). We report a patient with a severe form of CMT, with mutations in both genes and the molecular findings in 9 family members.

PATIENT: A 62-year-old woman with severe distal muscle weakness since childhood. The patient is wheelchair dependent since she was in the thirties. Electrophysiological studies revealed a sensory and motor neuropathy with mild demyelinating features and severe axonal degeneration. Analysis of GDAP1 revealed the mutation Gln95Stop in homozygous state. On the other hand, MFN2 analysis revealed the change Arg468His in heterozygous state. Clinical and Molecular analysis of eight family members shows two members with the MFN 2 change and no GDAP1 mutation, a 56-years-old male with a mild axonal form of neuropathy and his 18-year old daughter still without clinics. Three other members have the mutation in GDAP1 gene but in heterozygous state and no change in MFN2 gene. They have normal clinical and electrophysiological examinations. Duplication/deletion and point mutations in PMP22 and MPZ were ruled out.

#### P01.228

#### A case presentation: X-linked pattern of Charcot-Marie-tooth Disease

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A family with the presence of X-linked pattern of Charcot-Marie-tooth Disease .The proband is a woman who is coming after a consanguineous marriage for genetic counseling before pregnancy and her father has CMT disease and DM type 2. In the pedigree there are two male cases of CMT disease that are cousins of the proband case.

The clinical and paraclinic manifestations of the proband's father are: -A 50 years old man with muscular weakness and chronic paraparesis

-Paresthesia in lower extremities ,tingling and burning sensations of the feet  
 -Sensitivity of the lower extremities to the cold weather  
 -Gait problems  
 -Foot drop  
 -Pes cavus  
 -Hair loss of the lower limbs  
 -Tenar and hypotenar atrophy  
 -Loss of deep tendon reflexes  
 -Finger movement disorder in feet and hands  
 -High levels of LDH  
 -Chronic sensory-motor polyneuropathy in EMG and NCV.

#### P01.229

##### **MFN2 point mutations occur in 2% of Charcot-Marie-Tooth families - An investigation of 400 Norwegian CMT families**

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**Background.** Mutations in mitofusin 2 (MFN2) is the most common cause of Charcot-Marie-Tooth type 2 (CMT2).

**Methods.** Four-hundred Norwegian CMT families were screened for point mutations in the MFN2 gene.

**Results.** Of the 400 families eight had mutations in the MFN2 gene. We identified four novel point mutations located in exon 14, 15 and 18 (2 families). Clinically the known point mutations caused CMT2. The novel point mutations caused CMT2, distal Hereditary Motor Neuropathy (dHMN), intermediate CMT and CMT1 in each of the other families.

**Conclusions.** Our mutations broaden the clinical picture that can be seen with mutations in the MFN2 gene.

#### P01.230

##### **De novo point mutations in Cx32, EGR2, MFN2, MPZ, PMP22 and SIMPLE. A population based survey of Charcot-Marie-Tooth disorder**

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**Background.** An analysis of de novo mutations in persons residing in eastern Akershus County with Charcot-Marie-Tooth (CMT) disease.

**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 CMT patients were examined by geneticist and neurologist GJB. The Cx32, EGR2, MFN2, MPZ, PMP22 and SIMPLE genes were analyzed. Paternity tests were performed.

**Results.** We identified one de novo mutation in the MPZ gene and two de novo mutations in MFN2.

**Conclusion.** De novo point mutations are rare but less rare than previously anticipated.

#### P01.231

##### **Hereditary motor and sensory neuropathy type I in Russia**

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Hereditary motor and sensory neuropathy, also known as Charcot-Marie-Tooth (CMT) disease, is a large group of genetically heterogeneous hereditary disorders of distal nervous system. A group of 298 families with clinical and electrophysiological CMTI phenotype was investigated by us.

Mutations analysis was performed for three genes: PMP22, GJB1 and P0. First, the duplication in chromosome 17 (17p11.2-p12) was investigated by PCR-AFLP analysis of STR. Mutations analysis for PMP22, GJB1 and P0 genes was performed by direct automatic sequence on

Genetic Analyzer 3130 (Applied Biosystems).

Duplication of chromosome 17 was found in 189 families. This is 63,4% of all cases CMTI in Russian patients. GJB1 gene mutations caused of CMTI in 60 families, or 20,1%. Mutations in the P0 gene were found in 19 families, equaling 6,4%. Point mutation in the PMP22 gene were found in 2 families, that is 0,7%. In 28 families mutations were not found in any of these genes. Other genes, mutations of which lead to this disorder, will be analyzed in our next investigations.

#### P01.232

##### **The ARG94GLN mutation in MFN2 gene can be the cause of axonal Charcot-Marie-Tooth disease with optic atrophy**

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Some mutations in the mitochondrial GTPase mitofusin 2 (MFN 2) (MIM608507) have been related to the axonal form of Charcot-Marie-Tooth (CMT) disease with optic atrophy. We report a family with severe CMT disease and dominant inheritance. Electrophysiological studies revealed a severe sensory and motor neuropathy with conserved nerve conduction velocities (NCV). One of the family members presented a sub acute visual impairment. The ophthalmological studies revealed optic atrophy (OpA). Cranial Magnetic Resonance studies were normal. Molecular studies revealed the Arg94Gln mutation in exon 4 of MFN2 gene. This mutation had been previously reported, but only associated with "pure" axonal CMT and CMT with tremor. This is the first family with this mutation related to CMT with OpA reported, showing the importance of ophthalmological examinations in patients with axonal CMT caused by mutations in MFN2 gene.

#### P01.233

##### **Molecular study of MFN2 gene in spanish population**

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The axonal dominant form Charcot-Marie-Tooth type 2A (CMT2 A2) (MIM#609260) is caused by mutations in mitochondrial fusion protein mitofusin 2 (MFN2) (MIM608507). We report the molecular analysis of 101 families with suspected CMT 2. The 19 exons of the MFN2 have been amplified by polymerase chain reaction with the previously described primers by Zuchner et al. (2004) Amplified DNA samples were directly sequenced by applying the BigDye V3.1 (Applied Biosystems) and subjected to an capillary sequencer genetic analyzer. Duplication/deletion and point mutations of PMP22, connexin 32 and MPZ were previously ruled out.

The 83% of the families have been already studied. We have found 7 point mutations in 9 different families. The mutations were located in exons 4, 8, 9, 11 and 14. The study of the promotor region reveals one change of one nucleotide and two families with a deletion of 15 nucleotides. On the other hand, we have found 18 different polymorphisms. One of the found point mutations was not previously described. The frequencies of changes in MFN2 found in our population are similar to previously described studies; these findings confirm the importance of this gene in the physiopathology of the axonal type of CMT 2 A2 in our population too.

#### P01.234

##### **Dating the mutation Leu239Phe of the GDAP1 gene in CMT Russian families**

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Mutations in the ganglioside - induced differentiation-associated protein 1 (GDAP1) gene are common a cause of the Charcot-Marie-Tooth (CMT4A) disease with autosomal recessive mode of inheritance. To date more than twenty mutations in the GDAP1 gene have been reported in patients suffering from the demyelinating, axonal or mixed form of Charcot-Marie-Tooth disease.

In our study 110 patients from 72 unrelated families with CMT were screened for mutations by SSCP analysis with following direct sequencing of abnormal conformers.

A c.715C>T at substitution codon 239 (Leu239Phe) was detected in nine affected subjects from six apparently unrelated families. Allelic

frequency of this mutation averaged about 7% of all investigated chromosomes and 71% of chromosomes with mutations in *GDAP1* gene. Analysis of the *GDAP1* locus for markers D8S279-D8S1776-D8S286-D8S551-D8S548-D8S1805-D8S1705-D8S1757 demonstrated a common haplotype for markers D8S286; D8S551, D8S548, and D8S1805 on the chromosomes with c.715C>T mutation. The association of the mutation with a common haplotype suggested a common ancestor. The date of diffusion of the mutation has been calculated by linkage disequilibrium between disease locus and these polymorphic markers. The "age" of mutation c.715C>T in Russian was approximately 1000 years.

#### P01.235

##### Investigation of *GDAP1* Gene in Iranian CMT Patients

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Charcot-Marie-Tooth disease (CMT) is the most frequently occurring inherited peripheral neuropathy, affecting 1 in 2,500. The disease is characterized by distal muscle weakness and atrophy, predominantly involving the legs. CMT disease caused by mutations in the ganglio-sideinduced differentiation-associated protein 1 (*GDAP1*) gene is a severe autosomal recessive neuropathy originally reported in families with either demyelinating CMT4A neuropathy or axonal neuropathy with vocal cord paresis which maps to the CMT4A locus on chromosome 8q21.1. *GDAP1* is a 358 amino acid protein which expressed in both the central and peripheral nervous system. 22 Iranian families with a diagnosis of CMT disease, either axonal or demyelinating, were available for genetic analysis of *GDAP1*. Total genomic DNA was extracted from all family members using standard procedure. In all cases linkage analysis with different markers for the PMP22, MPZ, and GJB1 genes were used to exclude mentioned gens involving. In the 8 remaining families genotyping for the 3 microsatellite markers linked to the CMT4A locus was performed using different PCR protocols for each marker (D8S164, D8S286, and D8S551). PCR products were run on a 12% non denaturing polyacrylamide gel and allele fragments were visualised by silver staining. Our results showed the usefulness of linkage studies in diagnosis of CMT patients. We could identify and confirm CMT4A in 4 patients with use of these markers. The data in this study could also be used in prenatal diagnosis and carrier detection.

#### P01.236

##### Control population distribution and in silico functional analysis of novel genetic variants in Charcot-Marie-Tooth-Disease patients

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When a variation (uncertain variant, UV) is found in a disease candidate gene, it is critical to establish whether this change is neutral or responsible for the observed disorder. As a result of sequence mutation screening of *PMP22*, *MPZ*, *GDAP1*, *GJB1*, *EGR2*, *NEFL* and *LITAF* in a group of 47 Spanish patients with a clinical diagnosis of Charcot-Marie-Tooth disease we found three non-synonymous, four synonymous and five intronic nucleotide substitutions not contained in dbSNP. In order to assess the possible pathogenic role of these 12 UVs, two approaches were used:

1) Screening of 296 Caucasian controls, 200 of which are Galician individuals without any neurological disorder.

2) *In silico* analysis to explore:

- conservation across animal species (UCSC Genome Browser)
- the impact of an amino acid substitution on the structure and function of a human protein (Polyphen)
- a potential role as an exonic splicing enhancer (ESEfinder)

We present this strategy as a valuable mean to select the UVs most likely to have a biological function warranting further study by experimental models.

#### P01.237

##### Rapid diagnosis of CMT1A Deletion/duplication by real-time quantitative polymerase chain reaction

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Charcot-Marie-Tooth disease (CMT) is the most common form of hereditary motor and sensory neuropathy (HMSN). CMT has been classified into demyelinating (CMT1) and axonal (CMT2) forms.

Around 70% of CMT1A cases are caused by a dominantly inherited 1.5 Mb duplication at 17p11.2-12 encompassing the peripheral Myelin protein 22 (PMP22) gene. In contrast, hereditary neuropathy with liability to pressure palsies (HNPP) is caused by reciprocal deletion of the same 1.5 Mb region. In the present study, we developed a highly sensitive and specific quantitative gene dosage method for detecting the PMP22 duplication and deletion using Real time PCR. Real time quantitative PCR is a sensitive, specific and reproducible method for diagnosing PMP22 duplication and deletion. The method is fast, and requires no post-PCR handling.

#### P01.238

##### Molecular diagnosis of CMT1A and HNPP using Multiplex Ligation-dependent Probe Amplification (MLPA): Comparison with the PFGE-Southern blot analysis

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Background: Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) are the two most common peripheral neuropathies caused by a duplication or deletion of the 1.5-Mb region containing *PMP22* gene on 17p11.2, respectively. Although pulsed-field gel electrophoresis (PFGE)-Southern blot (SB) analysis is considered as the reference method for molecular diagnosis of CMT1A/HNPP, several methods such as fluorescence in-situ hybridization (FISH), short tandem repeat (STR) analysis, multiplex fluorescence PCR, and real-time PCR have been tried to avoid laborious and time-consuming PFGE-SB method.

Methods: We tried to evaluate newly developed multiplex ligation-mediated probe amplification (MLPA) method for the detection of the specific 1.5-Mb duplication/deletion by prospectively testing 31 patients referred for differential diagnoses of CMT1A or HNPP. MLPA probe-mixes contain *TEKT3*, *PMP22*, *FLJ25830*, *BX089850* and *COX10* genes within the CMT1A/HNPP region. The results with MLPA method were compared with our current PFGE-SB method.

Results: Thirteen out of 31 patients were diagnosed as having either duplication (n=3) or deletion (n=9) by PFGE-SB method and all the results were concordant with those by MLPA analysis. The turnaround time (TAT) by MLPA is estimated to be 4 days while TAT by PFGE-SB is approximately 17 days.

Conclusions: MLPA is a sensitive and specific technique for the detection of duplication or deletion of *PMP22* gene and its turnaround time is much shorter than the PFGE-Southern blotting. Therefore, MLPA could be a good alternative method replacing laborious PFGE-SB analysis.

#### P01.239

##### Subependymal heterotopia in the severe variant subtype of Adams-Oliver syndrome

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We recently ascertained a 7-year-old female born from non-consanguineous healthy parents with left talipes equinovarus, bilaterally absent/severely hypoplastic/malformed toes with absence of nails. Her psychomotor development was severely delayed and she suffered of seizures since the age of 3 years. At the time of examination (aged 13 years) she shows bilateral transverse reduction of digits, prominent veins over the trunk with rare café-au-lait spots and severe mental retardation with aggressive behaviour. Brain magnetic resonance scan shows bilateral nodular foci of tissue in the subependymal region lining the lateral ventricles. A focal area of irregular cortical surface is also present, suggesting cortical dysplasia.

This girl fits the diagnosis of Adams-Oliver syndrome (AOS) and cor-

roborate the existence of a clinically recognizable group of AOS patients with psychomotor delay, mental retardation, central nervous system (CNS) manifestations and seizures. Furthermore, subependymal heterotopia, never reported before in AOS, broadens the phenotypic spectrum of CNS abnormalities to include neuronal migration defects. This severe variant of AOS, whose phenotype has been recently delineated, is likely inherited in autosomal recessive fashion. Hence, the importance of a timely diagnosis not only to manage CNS manifestations but also to properly counsel the families.

#### P01.240

#### Aplasia Cutis Congenita type III, congenital scalp defects with distal limb reduction anomalies - Adams-Olivier syndrome.

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<sup>1</sup>Child Neurology Department, Silesian Medical University, Katowice 40-752, Poland, <sup>2</sup>Department of Molecular Biology and Genetics, Silesian Medical University, Katowice 40-752, Poland, <sup>3</sup>Department of Clinical Genetics, Memorial Children's Health Institute, Warsaw, Poland, <sup>4</sup>Student's Association at the Child Neurology Department, Silesian Medical University, Katowice 40-752, Poland. Aplasia cutis congenita (ACC) are the heterogeneous group of disorders with various clinical manifestations. Adams-Olivier syndrome (AOS, MIM 100300), a rare congenital disorder, belongs to the type III by Sybert classification of ACC, and is characterized by aplasia cutis congenita of the scalp and variable degrees of terminal transverse limb defects. Other associated anomalies include vascular defects: cutis marmorata, dilated scalp veins, small eyes, hypoplastic optic nerve, submucous cleft palate, cardiovascular and brain malformations (cortical malformations- polymicrogyria, encephalocoele, hypoplasia of the left arteria cerebri media).

Adams-Olivier syndrome is a genetic defect that causes a vasculopathy and leads to a variety of phenotypes. Inheritance of Adams-Olivier syndrome is autosomal dominant but there are also reports of possible autosomal recessive mode of inheritance.

The authors present a 27-month-old-boy with phenotype of Adams-Olivier syndrome and Peter's anomaly (central leukomata with variable iridocorneal and keratolenticular adhesions and cataract), refractory epilepsy and psychomotor retardation. The molecular investigation is ongoing in order to identify the genetic cause of AOS.

#### P01.241

#### Manifestation of X-linked Adrenoleukodystrophy in heterozygous carriers

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X-linked adrenoleukodystrophy (ALD, MIM 300100) has several phenotypes, clinical manifestation in women-heterozygous carriers among them. About 15-20% of carriers develop neurological signs, typically lower spastic paraparesis resembling adrenomyeloneuropathy (AMN) without adrenal insufficiency. After 40 yrs this proportion amounts 50%. Though, ALD manifestation in women is often misdiagnosed. In our sample of 50 unrelated families with verified ALD there were two families with evident manifestation in women. Low proportion of these cases may be partly due to the young age of most mothers in the sample. In one family, 7-year-old proband had childhood cerebral ALD, in his younger brother presymptomatic stage of disease was found out. Their 26-year-old maternal uncle showed typical presentations of AMN, 52-year-old grandmother had progressive spastic paraparesis since 35-40 yrs. Prior to ALD discovering in the proband they had diagnosis of Strümpell's disease despite evident adrenal insufficiency in the uncle. In 3-year-old proband from another family ALD manifested by adrenal insufficiency as early as in 2.5 yrs, brain MRI proved childhood cerebral form. His 32-year-old mother and her monozygotic twin since 27 yrs suffered spastic paraparesis with bladder disturbances and normal MRI, their previous diagnosis was multiple sclerosis. Such cases along with reported earlier [Krenn et al, 2001; O'Neill et al, 2001; Shaw-Smith et al, 2004] point that women suspicious for Strümpell's disease or familial multiple sclerosis should be tested for ALD if pedigree permits X-linked inheritance. Both families, particularly the first one, also support the known fact of different ALD phenotypes intrafamilial co-existence.

#### P01.242

#### New mutations and sequence variations in galician patients with familiar Alzheimer's Disease

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Mutations in presenilin 1 (*PSEN1*) are detected in 30-70% of familiar Alzheimer disease (FAD) while presenilin 2 (*PSEN2*) and amyloid precursor protein (*APP*) mutations are much less frequent. We studied 61 clinical-based cases with a diagnosis of probable FAD. We sequenced all coding exons of *PSEN1* and *PSEN2*, as well as exons 16 and 17 of *APP* on an ABI3730 sequencer. Sequence analysis was performed with the Staden package. A novel L424V mutation was identified in exon 12 of *PSEN1* in a patient with very young onset dementia. Another new mutation, I408T, was identified in exon 11 of *PSEN1* in a family. In two other cases of FAD missense mutations were detected in *PSEN2*: A415T and R435Q, both in exon 12. Five patients showed intronic changes of unknown significance not reported in the searchable databases of human polymorphisms. We identified the single nucleotide substitution c.338+39G>A in intron 4 of *PSEN1*, a 6 bp deletion in intron 17 of *APP* and three intronic variants in *PSEN2*: c.119-31G>A (intron 4), c.887-19A>C (intron 8), c.1191+57T>A (intron 11). A synonymous change (S236S) in *PSEN2* was present in two individuals. All new mutations and sequence variations were screened in a panel of 186 Galician control individuals without neurological disorders. Globally mutations were detected in 6.6% of the cases (18% if we consider both missense mutations and sequence variations of unknown significance). This low frequency of mutations may be due to not very restrictive referring criteria. Alternatively, other genes may explain most FAD in our region.

#### P01.243

#### Left Ventricular Outflow Tract Obstructions (LVOTO): family data and NOTCH1 mutations

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Left ventricular outflow tract obstructions (LVOTO) are often familial. Early detection of (latent) LVOTO or increased familial risk can prevent unexpected cardiac death. To analyse the percentage of familial cases all new and known patients with left sided anomalies seen after April 1<sup>st</sup> 2006 by the department of pediatric cardiology (170) were offered genetic counselling. Thirty-one patients refused genetic counseling, the others were seen by the same clinical geneticist. In 44 an aortic valve stenosis was diagnosed, in 21 a bicuspid aortic valve without stenosis, in 28 a hypoplastic left heart, in 67 an aortic coarctation and in 10 other left sided anomalies. In 17 patients the family history combined with ultrasound of first degree relatives revealed a LVOTO and in another 19 probands a relative with yet another congenital heart defect was found.

The first mutations in *NOTCH1* were published in 2005 by Garg et al. in two families with bicuspid aortic valve and other heart defects, and so far two small series showed mutations in approximately 4% of the patients. In our patient group, sequencing has been finished in 40 patients, and 3 mutations have been found.

We conclude that LVOTO is often familial and most pedigrees are compatible with autosomal dominant inheritance with incomplete penetrance. *NOTCH1* mutations are found in a small percentage of familial and non-familial cases.

#### P01.244

#### Asymptomatic neurocutaneous melanosis: A case report and review of literature

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Neurocutaneous melanosis (NCM) is a rare congenital syndrome characterized by the presence of Giant Congenital Melanocytic Nevi (GCMN), either multiple Smaller Congenital Melanocytic Nevi (SCMN), or both. NCM has characteristically been reported to manifest early in life, usually by age 2 to 2.3 years of age and the incidence for asymptomatic NCM with abnormal MRI is 4.8%. The Proposita is a 2 years-old, Hispanic female, was the product of the 5th, full-term and uncomplicated pregnancy; from non-consanguineous parents. She has a history of Giant Congenital Nevi and several episodes of hydrocephaly. Clinically, she has Psychomotor delayed; macrocephaly, hydrocephalus; hemangiomas, multiple congenital pigmented nevi and a nevus approximately 38 x 30 cm. that covers the lumbosacral area, lower abdomen and both thighs. This nevus is dark brown and black colored, with irregular borders; and is covered by thin hair in some areas. Face, neck, arms, chest, upper back and abdomen are covered with multiple satellite melanocytic nevi, which measure 1 to 5 cm. in their largest diameter. Palms and soles are not affected. The CT scan showed dilatation of all ventricles; MRI with gadolinium reported severe hydrocephalus and there were non-hyperintensity regions. We concluded that the patient has NCM associated to GCMN and numerous congenital melanocytic nevi.

#### P01.245

#### A deficit of ATPase subunit 8 : with contribution for two new cases

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In two consanguineous children ,brother and sister, was reported a rare mitochondrial disorder, still not described in literature, caused by mutation of the gene MT-ATP8: base change T8412C with aminoacid change : methionin-threonin which lead to dolichocephaly, protruding metopic suture, muscle hypotonia , ataxia and mental retardation.The EEG showed diffuse changes of tetra type.The CAT showed atrophy of the brain of the first child.The investigation of the mother showed the same mutation which will help the prenatal diagnosis .A treatment was started with high doses of vit.B1, B6, B12, L-carnitine, coenzyme Q10 with the aim to stimulate the enzyme processes and compensatory to increase the ATP by alternative metabolic pathways.The clinical investigation continues.

#### P01.246

#### X-linked α thalassaemia/mental retardation syndrome - a case with gonadal dysgenesis, caused by a novel mutation in ATRX gene

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X-linked α thalassaemia/mental retardation (ATR-X) syndrome is one of the genetic conditions that results in X-linked mental retardation. Its principal features are: learning difficulties, microcephaly with characteristic facial features (hypertelorism, nose with flat bridge and triangular, upturned tip, full, everted lower lip) and α thalassaemia. Other known traits of ATR-X are genital abnormalities, the severity of which varies from cryptorchidism to ambiguous genitalia. We present a family referred to the genetic service with suspicion of Smith-Lemli-Opitz (SLO) syndrome after the birth of a child with facial dysmorphisms, atrioventricular defect and ambiguous genitalia. Unfortunately, he died before diagnostics. In a subsequent pregnancy, chromosomal analysis showed the foetus had a normal male karyotype (46, XY) and SLO syndrome was also excluded. However, at birth the baby exhibited the same spectrum of clinical features as in the previous child. Amongst

other abnormalities, external female genitalia and dysgenetic testes were detected. He was screened for subtelomeric aberrations but no rearrangements were identified. Observed genital anomalies, together with the distinct facial appearance and the presence of Haemoglobin H inclusions in the red blood cells, led to a diagnosis of ATR-X syndrome. Further molecular study revealed a novel mutation in ATRX (c.G590-T, p.Cys197Phe), coding for a change from a highly conserved cysteine to a phenylalanine residue. The same mutation was identified in the mother, so we can speculate that her elder child was also affected with ATR-X syndrome.

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#### P01.247

#### CADHERIN-11 as a possible candidate gene for autism

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#### Background:

Positional cloning of chromosomal translocation breakpoints in autism patients is a valuable strategy towards the identification of candidate genes, especially in isolated autism, and when the aberration is de novo and family history is negative for autism.<sup>(1)</sup>

#### Objectives:

We describe a nondysmorphic patient with autism and full scale IQ of 76, who carries a complex translocation involving chromosomes 3,5,16 and a pericentromeric inversion on chromosome 4.

#### Methods:

1Mb BAC array-CHG was performed to uncover possible submicroscopic imbalances. The breakpoints were finemapped with FISH.

#### Results:

On array-CHG, a ~1Mb microdeletion encompassing the clone NON-SC8G10 was detected. Only 1 gene, cadherin-11 (*CDH11*) was located in this region. Of the remaining 6 breakpoints, one disrupted the *AK13094* gene, another was near *GFOD* and *RANBP10*.

#### Conclusions:

In this patient with a complex chromosomal aberration, the *CDH11* gene was found to be heterozygously deleted. *Cdh11* is expressed in the limbic system and hippocampus in mice and may play a role in the organisation of central synapses.<sup>(2)</sup> Interestingly, there is evidence that altered synaptogenesis is implicated in the pathogenesis of autism. For instance, the cell adhesion molecules *NLGN-3* and -4 are implicated in autism through their role in the establishment of functional presynaptic terminals in contacting axons.<sup>(3)</sup> Thus, *CHD11* represents both a positional and functional candidate gene for autism. Mutation screening in a larger cohort of autism patients is ongoing.

<sup>(1)</sup> Castermans (2003) J Med Genet 40(5) 352-6

<sup>(2)</sup> Manabe (2000) J Mol Neurosci 15(6) 534-46

<sup>(3)</sup> Persico (2006) Trends Neurosci 29(7) 349-58

#### P01.248

#### FMR1 gene: prevalence of premutation and intermediate/grey zone alleles in an autistic Basque sample

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Fragile X Syndrome (FXS) is associated with an unstable CGG repeat sequence in the 5' untranslated region of the first exon of the FMR1 gene. The location of this gene coincides with a Fragile Site (FS) FRAXA. The CGG sequence is polymorphic with respect to size and purity of the repeat. Although only the full mutation (>200 CGG) is associated with clinical expression of FXS, premutation (55-200 CGG) and intermediate/grey zone allele carriers (35-54 CGG) have been also associated to distinctive phenotypes, one of these are mental retardation and/or autism. Autism is a behavioral disorder of early onset marked by social and cognitive deficiencies. Our Previous studies of chromosomal fragility in autistic and normal individuals show higher frequency of FS and FRAXA full mutation in autistic individuals. The

aim of this study was to analyze a sample of autistic patient without FRAXA full mutation to know the correlation of premutation and intermediate/grey zone size FMR1 alleles and autism. The results show that the prevalence of premutation alleles is 2/10 in patients, however we did not find intermediate/grey zone alleles. Beside the overall repeat size and the AGG interspersion pattern suggest instability.

#### P01.249

#### **Autosomal dominant Alport syndrome: molecular analysis of the COL4A4 gene and clinical outcome**

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Alport syndrome is a clinically and genetically heterogeneous nephropathy characterized by irregular thinning, thickening, and splitting of the glomerular basement membrane often associated with hearing loss and ocular symptoms. While the X-linked and the autosomal recessive forms are well known, the autosomal dominant form is not well acknowledged. We have investigated 37 patients with a clinical and molecular diagnosis of autosomal ATS belonging to 8 different families. The mean age of patients was 38.7 years ranging from 6 to 76 years. Only 9 out of 37 (24.3%) patients reached the ESRD, at the mean age of 51.2 years. Four patients (13.8%) had hearing loss and none ocular changes. DHPLC analysis revealed 8 novel private COL4A4 gene mutations: 3 frameshift, 3 missense and 2 splice-site mutations. These data indicate autosomal dominant Alport syndrome as a disease with a low risk of ocular and hearing anomalies but with a significant risk to develop renal insufficiency although at an older age than autosomal recessive and X-linked forms.

These clinical features make difficult differential diagnosis with the benign familial hematuria due to heterozygous mutations of COL4A4. On the other hand, we are unable to demonstrate a genotype-phenotype correlation with the type and the site of the COL4A4 mutations. A correct diagnosis and prognosis is based on a comprehensive clinical investigation in as many family members as possible.

#### P01.250

#### **COL4A1 mutation in Axenfeld-Rieger anomaly with leukoencephalopathy and stroke**

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INTRODUCTION : Axenfeld-Rieger anomalies (ARA), refer to a wide variety of abnormalities of the anterior segment of the eye that belong to the heterogenous family of the anterior segment dysgenesis. ARA appears to be genetically heterogeneous: it has been associated with mutations in three genes: PITX2 (on chromosome 4q25), FOXC1 (also named FKHL7) (6p25), and PAX6 (11p13).

SUBJECTS AND METHODS : Five members of a three-generation family affected by vascular leukoencephalopathy and ARA, were clinically and genetically investigated.

RESULTS : Diffuse leukoencephalopathy associated with ocular malformations of the Axenfeld-Rieger type was observed in the 5 affected individuals. Genetic linkage analyses directed toward three loci associated with hereditary vasculopathies and on the four loci known to be associated with ARA showed a possible association with the two loci 11p13(PAX6) and 13q34 (COL4A1). Direct sequencing of the COL4A1 gene led to the identification of a novel missense mutation p.G720D, which cosegregates with the disease.

CONCLUSION : We delineate a novel association between the Axen-

feld-Rieger anomaly and leukoencephalopathy and stroke. Our data confirm that mutations in the COL4A1 gene can be responsible for ARA in humans, as suggested by previous studies in *Col4a1*-mutated mice.

#### P01.251

#### ***De novo balanced chromosomal translocation (2;10)(q11.2;q24) associated with dysmorphic features and mental retardation. Case report.***

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We report a clinical case of a reciprocal translocation between the long arms of the 2<sup>nd</sup> and 10<sup>th</sup> chromosomes observed in a 12 years old male with dysmorphic features, mental retardation and compulsive - obsessive behaviour.

From anamnesis: boy was born from uncomplicated 1<sup>st</sup> pregnancy from nonconsanguineous marriage. Delivery on 40<sup>th</sup> week of gestation, birth weight 3450g, height 58cm. At the age of 6 months he had first episode of seizures after injury of the head. Seizures repeated two times till 12 months of age, later they have never been observed. Motor and speech development were normal. Behavioural problems first noticed at 4 years of age.

Psychiatric findings: marked hyperactive behaviour, always in motion, restless, enthusiastic, frequently clownish, mincing, dyslalia and echolalia, might be aggressiveness to others and to himself, moderate mental retardation.

Dysmorphic features: downslanting palpebral fissures, facial asymmetry, left eyelid ptosis, hypotelorism, esotropia, and irregular tooth placement.

There was performed a conventional GTG karyotyping. Result: 46,XY, t(2;10)(q11.2;q24). Chromosomal breakpoint was confirmed with FISH (Kreatech MYCN (2p24) & LAF (2q11)) analysis.

It still remains a question of discussion if balanced translocation could be associated with dysmorphic features and mental retardation. To evaluate possibility of certain genes loss in breakpoint regions, further SNPs analysis of regions is in progress.

#### P01.252

#### **Bardet Biedl Syndrome Analysis Among Iranian Families**

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Bardet-Biedl syndrome (BBS) is characterized by cone-rod dystrophy, truncal obesity, postaxial polydactyly, cognitive impairment, male hypogonadotropic hypogonadism, complex female genitourinary malformations, and renal dysfunction. Birth weight is usually normal, but significant weight gain begins within the first year and becomes a lifelong issue for most individuals. A majority of individuals have significant learning difficulties, but only a minority has severe impairment on IQ testing. Renal disease is a major cause of morbidity and mortality. In our own study the initial diagnosis of Bardet-Biedl syndrome is established by clinical findings in our specialists' thorough exam.

Twelve genes are known to be associated with Bardet-Biedl syndrome: BBS1, BBS2, ARL6/BBS3, BBS4, BBS5, MKKS/BBS6, BBS7, TTC8/BBS8, B1/BBS9, BBS10, TRIM32/BBS11, and BBS12. Indirect molecular diagnosis using Bardet-Biedle markers facilitate prenatal diagnosis of Bardet-Biedl children.

During the period of 2007 all referred patients to our lab have undergone molecular genetics analysis. Five molecular markers: D11S913, D16S408, D3S1254, D15S131, D4S402 are used for covering the analysis according to the previous surveys.

The primary results showed that in all families' segregation of alleles are according to mendelian inheritance and affected chromosomes are distinguishable from unaffected ones. The patients' molecular genetics profile has been completed through PCR amplifications and Electrophoresis in PAGE gel and the final linkage analysis showed that BBS4, BBS7 were the most associated gene with the Bardet-Biedl in Iranian families.

**P01.253****The first case of Berardinelli-Seip congenital lypodystrophy reported in Lithuania**

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Berardinelli-Seip congenital lypodystrophy (BSCL) syndrome is a extremely rare autosomal recessive disorder, with estimated prevalence of 1 in 10 million population. Two genes are known to be associated with BSCL: *AGPAT2* and *BSCL2*. Individuals with mutations in *BSCL2* include whites of varying ethnicities originated mostly from Europe and Middle East, and with mutations in *AGPAT2* typically originate from sub-Saharan Africa and Maghreb.

**Case report.** Our presented patient, male, was the first child of non consanguineous parents Lithuanian origin. BSCL syndrome was diagnosed one month after birth by the phenotypic characteristics of the syndrome. At the age of the 9 years patient had total loss of subcutaneous fat, muscular hypertrophy, long extremities, acromegalic appearance, dry and curly hair, umbilical hernia, hyperhidrosis, distinct acanthosis nigricans, marked hepatosplenomegaly, divergent alternant strabismus, hypermetropia, slight mental retardation. Patient's pubertal status was stage 2 according to Tanner's charts, the bone age was of 14 years. His blood pressure was slightly increased, he had increased fasting glucose, dyslipidemia, mild proteinuria, marked hyperinsulinemia, increased hepatic enzymes, signs of left ventricular hypertrophy. Liver biopsy showed steatohepatitis of low activity and hepatic fibrosis. Mutation analysis revealed that our patient was compound heterozygous for two mutations of *BSCL2* gene [c.458C>A]+[c.412C>T] (testing was performed in Belgium, Loverval, Human Genetic Centre). The results confirmed the clinical diagnosis of BSCL and allowed assignment to type 2. To our knowledge this is the first report of Berardinelli-Seip syndrome in Lithuania.

**P01.254****Biallelic BRCA2 -the first Greek Cypriot Family reported**

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MM was born with multiple congenital anomalies, affecting mainly her growth, limbs, digits and kidneys. She also had short stature, microcephaly and dysmorphic facial features. Her right thumb was ectopic whereas her left thumb was absent. Fanconi anemia (FA) was suggested.

Chromosomal examination revealed anomalies (end to end fusion, deletions and chromatid breaks) also compatible with the clinical diagnosis of FA. MM was diagnosed with metastatic neuroblastoma at six months of age and passed away a month later.

Parents had three miscarriages. At the 24th week of their fifth pregnancy (in vitro fertilization) fetus was found to be microcephalic and were referred back for genetic counselling. Based on family history of breast cancer both parents were tested for BRCA mutations and found to be positive for the BRCA2 mutation 8984DelG which has been reported as a founder mutation among Greek Cypriots. DNA stored from MM was tested and found homozygous for BRCA2 alleles. The FA phenotype related to biallelic BRCA2 is associated with high spontaneous chromosome aberration rate, less frequent bone marrow suppression and a different spectrum of childhood cancers. Cancer risk for children with biallelic BRCA2 mutations may be very high. This is the first family diagnosed among Greek Cypriots with biallelic BRCA2 mutations manifesting breast cancer and FA.

**P01.255****Expansion and diversity of the phenotype and results of molecular studies in patients with blepharocheliodontic syndrome**

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Blepharocheliodontic (BCD) syndrome is one of the at least 400 orofacial clefting syndromes known, first described by Elschnig in 1912 and recognised as a syndrome and named by Gorlin in 1996. It is a rare, but possibly underdiagnosed, autosomal dominant condition with variable expressivity in which the main clinical features are orofacial clefts, ectodermal defects and ocular abnormalities. The ectodermal defects are mainly oligodontia, delayed dentition, conical crown form, hair abnormalities (pili torti, sparse hair, eyebrows and eyelashes) and hypodysplastic nails. The ocular abnormalities comprise ectropion of the lower eyelid, distichiasis of the upper eyelid, euryblepharon and lagophthalmos. Other dysmorphic features have been described in BCD patients, like hypertelorism, ptosis, broad forehead with high frontal hairline, dysplastic ears, clinodactyly and syndactyly. Also ankyloblepharon, hypothyroidism, dermoid cysts and membranous imperforate anus were suggested to be part of the spectrum.

While BCD and lagophthalmos syndrome are a continuum, as suggested by Gorlin, BCD syndrome shows clinical overlap with ectrodactyly-ectodermal dysplasia-cleft lip/palate (EEC) syndrome, ankyloblepharon-ectodermal defects-cleft lip/palate (AEC) or Hay Wells syndrome and van der Woude/popliteal pterygium syndrome. A gene for BCD syndrome has not yet been identified and might be involved in neural crest and/or branchial arch development.

The variable clinical features of two families comprising nine patients and three other sporadic patients with BCD syndrome will be presented and compared to the patients described before. It will be discussed if the phenotype can be expanded with neural tube defects. Also the results of molecular studies in these patients will be shown.

**P01.256****A three generation Serbian family with C263T mutation in MPZ gene**

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The myelin protein zero (MPZ) gene encodes an integral membrane protein with immunoglobulin-like extracellular domain. MPZ is expressed only in peripheral nerves, and is localized in compact myelin. Mutations in MPZ gene are the common cause of peripheral neuropathies. Depending on the location and type of the mutation, MPZ is associated with demyelinating either with axonal phenotypes. Mostly, mutations that lie in extracellular domain of MPZ and affect tertiary structure of the protein important for myelin sheet formation lead to demyelinating neuropathies, and mutations affecting myelin-axon communication induce axonal degeneration.

Here we present a three generation family with C263T mutation that affects extracellular domain of MPZ.

Clinical features are listed in a table:

Patient	Age (years)	Gender	Age at onset of symptoms of weakness (years)	Motor nerve conduction velocity (MNCV) for median nerve (m/s)
II1	65	F	Unknown	Not done
II2	47	F	>40	22
III1	40	M	11-20	35.7
III2	38	F	6-10	17.6
III3	20	F	11-20	22
IV1	7	F	/	18.5

Mutations causing peripheral neuropathies show intra- and interfamilial variability. In presented family MNCVs are severely reduced, except for the only male patient, whose MNCV is 35.7 m/s and could fit even in a group of axonal neuropathies. There is no extensive intrafamilial variability in this family, but, the only male patient displays milder symptoms than other, female patients.

Although, there were no previously reported association between MPZ gene mutations, sex and the severity of the disease, cases like this should be recognized in order to remind us that our knowledge of biology of myelin sheet and peripheral nervous system is limited and, probably, more complex than we assume.

**P01.257****S248F mutation in CHRNA4 gene has a pathogenic role in autosomal dominant nocturnal frontal lobe epilepsy****A. Zuniga, E. Pineda, I. Pitarch, A. Guerrero;***Hospital de la Ribera, Alzira (Valencia), Spain.*

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE, OMIM 600513) has long been misdiagnosed as nocturnal paroxysmal dystonia, parasomnias, or psychiatric disturbances. It is characterized by clusters of brief nocturnal motor seizures, which originate in the frontal cortex. The results of interictal electroencephalogram are usually normal, as are computed tomographic scans and magnetic resonance images. Seizures are usually well controlled by carbamazepine; however, the recurrence risk after drug withdrawal often persists for life. This syndrome is inherited as an autosomal dominant disorder with a penetrance of 70% to 80%. Mutations in the alfa-4 subunit of the neuronal nicotinic acetylcholine receptor gene (CHRNA4) has been described in several families as responsible of ADNFLE.

We have studied a family with ADNFLE from the east of Spain. The clinical appearance of the disease in this family was very similar to previously described cases in which mutations in exon 5 of the CHRNA4 gene were found. So, the exon 5 of the CHRNA4 gene was amplified between nucleotides 535 and 825 and polymerase chain reaction products were purified and sequenced directly. We have identified in three affected members of family a identify a C-to-T transition in the CHRNA4 gene, resulting in a Ser-248-to-Phe (S248F) substitution in the sixth amino acid position of the transmembrane domain 2 (M2). Some authors have suggested that the mutation caused reduced receptor function. These data support the hypothesis that the phenotypic expression of autosomal dominant nocturnal frontal lobe epilepsy is caused by mutations in the CHRNA4 gene.

**P01.258****Further delineation of the partial trisomy 3q phenotype****D. Mueller<sup>1</sup>, W. Mueller<sup>2</sup>, F. Fresser<sup>1</sup>, G. Utermann<sup>1</sup>, D. Kotzot<sup>1</sup>;**<sup>1</sup>*Division for Clinical Genetics, Innsbruck, Austria, <sup>2</sup>Department of Pediatrics, Reutte, Austria.*

Partial trisomy 3q is characterized by postnatal growth retardation, microcephaly, developmental delay, and distinct facial dysmorphisms. Here, we report on a 3 years and 7 months old girl with minor dysmorphic features (high frontal hairline, down-slanting palpebral fissures, telecanthus, epicanthus, bulbous nasal tip, long philtrum, tapering fingers, and short 5<sup>th</sup> fingers) and language accentuated development delay due to partial trisomy 3q and partial monosomy 4p born to healthy parents (mother 20 years, father 33 years). After an unremarkable pregnancy, she was spontaneously delivered at term. Weight was 3920g (>90th percentile), length was 51cm (50-75th percentile), and OFC was 36.5cm (>90th percentile). At the age of 39 months height was 100cm (50-75th percentile), weight was 17.3kg (90-97th percentile), and OFC was 52cm (97th percentile).

GTG-banding and FISH with subtelomeric probes (Total Telomer Probe Panel, Vysis<sup>®</sup>) and a probe specific for the Wolf-Hirschhorn syndrome critical region (WHSCR) were performed according to standard procedures and revealed a de novo trisomy 3q27->qtel and monosomy 4p16->p1tel with a breakpoint distal to the WHSCR and proximal to the subtelomeric 4p probe (karyotype: 46,XX,der(4)t(3;4)(q27;p16).ish der(4)t(3;4)(D3S4560+;D4S3359-,WHSC1+CEP4+)). For a more exact breakpoint determination SNP-array analysis is underway.

In accordance with the cytogenetic results the girl shows no clinical features of WHS, but facial dysmorphisms clearly resembling partial trisomy 3q. The lack of postnatal growth retardation, microcephaly, and malformations in our patient will help to improve genotype-phenotype correlation in partial trisomy 3q.

**P01.259****Optimizing clinical diagnosis for some genetic syndromes with cleft lip and/or palate (Iasi Medical Genetics Center's experience)****E. Braha, M. Volosciuc, C. Rusu, M. Covic;***University of Medicine and Pharmacy, Iasi, Romania.*

Clefts of the lip (CL) and/or palate (CP) are among the most common birth defects and require multidisciplinary approach for diagnosis and treatment. The purpose of this report is to optimizing the clinical diagnosis of some genetic syndromes for the patients with CL/P. We selected 106 patients (58 boys and 48 girls) with CL/P from the 8615 total

patients evaluated in Iasi Medical Genetics Centre in 2000-2004. The most patients (47.2%) had CLP (19.8% bilateral) or a CP (47.2%); only 5.66% had CL. We look at other anomalies associated with CL/P, associations which have a high power to suggest the diagnosis. We used diagnosis algorithms and POSSUM, OMD databases. The 52.83% (56 patients) had CL/P associated with syndromes that include anomalies involving multiple organs. Only 47.17% were non-syndromic. Base on evocative signs the syndromes diagnosed in our selected cohort were: oculo-auriculo-vertebral spectrum (6), velocardiofacial syndrome (6), chromosomal anomalies (5), amniotic bands (3), Smith Lemli Opitz (3), EEC (3), holoprosencephaly (2), fetal alcohol (2), Aarskog (2), other rare syndromes (18). For accurate assessment, correct diagnosis, and management, the patients should be dealt with in a team approach. CL/P are frequent associated with others anomalies which emphasize the importance of plurimaleformative syndromes identification. Clinical proper diagnosis is essential to initiate genetic test, the correct management and genetic counselling.

**P01.260****Bannayan-Riley-Ruvalcaba syndrome confirmed by mutation in PTEN gene****R. Zordania, A. Lehtmetz, H. Pöder, K. Joost;***Tallinn Children's Hospital, Tallinn, Estonia.*

Bannayan-Riley-Ruvalcaba (BRRS) and Cowden (CS) syndromes are autosomal dominant allelic multiple hamartoma syndromes, both in 60-80% cases due to germline mutations in tumor suppressor *PTEN* gene. Clinical signs in childhood have many similarities, early diagnosis using molecular genetic analysis is important as syndromes give increased risk for different tumors.

We describe a family, where both young parents of our index patient were treated due to tumors- mother had bladder carcinoma, treated during pregnancy and father was treated due to embryonal testicular carcinoma.

Proband is the first and only child in the family. His birth anthropometry (4020g/52cm, OFC 41cm) was above 97th centile. During the first two years of his life he had psychomotor developmental problems, hypotonia, seizures with EEG changes, autistic features, unilateral hydrocele and macrocephaly. Metabolic workshop excluded metabolic disorders.

At the age of three years three lipomas were (histologically proved) diagnosed- two subcutaneous and one pancreatic. The patient had different skin symptoms, no pigmentation of the glans penis. He had macrocephaly and mild mental retardation and some autistic features and speech problems.

Mutation analysis of *PTEN* gene was performed by Dr.D.O.Robinsin (Salisbury Health Care NHS Trust). The patient is carrying a C>A substitution at base 144, in exon 2 of the *PTEN* gene. This mutation impairs the function of *PTEN* and is the cause of the patient's symptoms.

**Conclusion:** clinical, pedigree data and results of moleculargenetic investigations of the patient having Bannayan-Riley-Ruvalcaba syndrome are presented. This is the first case of this syndrome in Estonia confirmed *PTEN* gene mutation.

**P01.261****Report of cockayne syndrome from Iranian families****F. Afrozian, N. Almadani, Y. Shafeeghati, M. H. Karimnejad;***Karimnejad-Najmabadi Pathology and Genetics Center, Tehran, Islamic Republic of Iran.*

Cockayne Syndrome is an autosomal recessive multisystemic condition,characterized by usually senile-like changes beginning in infancy.

Retinal degeneration,Impaired hearing and photosensitivity of thin-skin.

The disease is known to be genetically heterogeneous for which 3different Loci have been identified on chromosomes 10,12,13. We have studied five Iranian families,each with one affected child(threemale,two female).three of these cases are results of consanguineous marriage and the parents of the two cases are offspring of unrelated couples.Their features and radiology were compatible with Cockayne Syndrome.

The ophthalmologist showed salt&pepper retinal pigmentation for two of the affected children and optic atrophy for other three cases.

Assays of DNA repair are performed on skin fibroblasts.The most

consistent finding in Cockayne Syndrome, fibroblasts, marked sensitivity to UV radiation, deficient recovery or RNA synthesis following UV damage (and impaired repair of) activity transcribed genes, or transcription couple repair.

PND of CS has been reported by analysis of UV light sensitivity and DNA repair in fetal cells obtained by CV or Amniocentesis.

#### P01.262

#### Complete androgen insensitivity syndrome within a large Turkish family from Southeast of Anatolia

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Complete androgen insensitivity syndrome (CAIS) due to inactivating mutations of the androgen receptor (AR) is an androgen receptor function disorder. Here, we present a large family with 6 children out of which 4 was CAIS affected. Cytogenetic analysis of the two healthy sibs revealed one brother (46,XY), one sister (46,XX) and four affected sisters with a normal male karyotype: 46,XY. The mother, the father and 5 sisters were tested with STR markers from X and Y chromosomes to evaluate the origin of X chromosomes in affected versus non-affected siblings. AMEL, XE1 (DXS6803), XE3(DXS6809), XHPRT(DXS6854), X22 (DXS8377) markers were used for the X chromosome and SRY, AMEL, YE4 markers were used for the Y chromosome. In all affected CAIS sisters, an X pattern similar to one of the two X chromosomes from the mother was observed and the Y markers correlated well with the father's Y markers as expected. The unaffected sister did not possess the relevant X haplotype; thus, she was presumed not to be a carrier. Lately this unaffected girl is reported to have two healthy daughters.

**Key words:** Complete androgen insensitivity syndrome, large family, STR markers, PCR.

#### P01.263

#### Evaluation and management of patients with complex chromosomal abnormalities

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We present 5 cases, apparently simple, but with complex chromosomal abnormalities on the karyotype, in order to discuss the importance of the cytogenetic evaluation for the management of the case and the genetic counselling offered to the family.

Case 1: male, 7 years old, first child of an apparently healthy, young, unrelated couple, who had also a miscarriage. The mother was pregnant again. Physical examination: typical aspect of Down syndrome. Karyotype: 46,XY,-13,+rob(13;21)/47,XXY,-13,+rob(13,21). Mother: carrier of the robertsonian translocation, as well as the fetus.

Case 2: male, 6 month old, third child of an young, unrelated couple. The mother has been diagnosed with syphilis during the pregnancy. Physical examination: mild aspect of Down syndrome. Karyotype: 47,XY,t(1;2)(p32-pter;q37-qter),-3,-21,+der(3)rcp(3;21)(p11.1;q22.2),+der(21)rcp(3;21)(p11.1;q22.2),+21. Parents: normal karyotype.

Case 3: female, 7 years old, first child of an apparently healthy, young, unrelated couple, that has also a healthy son. Physical examination: mild aspect of Turner syndrome. Karyotype: 44,X,der(13;14). Father: carrier of the robertsonian translocation.

Case 4: male, 5 years old, first child of an apparently healthy, young, unrelated couple, that has also a healthy son. Physical examination: typical aspect of trisomy 8. Karyotype: 47,XY,+8/47,XY,+8q/46,XY/45,X. Parents: normal karyotype.

Case 5: female, evaluated due to fertility problems. Karyotype: 45,X,inv9/47,XXX,inv9.

The mechanism that led to the complex chromosomal abnormality, as well as the clinical picture, the management and genetic counselling are discussed for all cases.

In conclusion, we present 5 cases of complex chromosomal rearrangements to illustrate particular situations (apparently simple cases, but with complex karyotype) and to discuss genetic counselling in these situations.

#### P01.264

#### Importance of early track down of congenital cardiac malformations

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**Introduction:** Congenital malformations are the main cause of death in the first year of life; therefore, early identification of etiology and right therapeutic decision are vital problems in pediatric services.

**Objectives:** genetic consult and family investigation; examination, investigation and selection of cases which require special methods of diagnosis, interdisciplinary consult, adaptation of European pattern for the anomalies management.

**Material and method:** The study includes 187 children with age of 0-1 year, consulted, hospitalized and investigated in Premature and Neonatology Department of Clinical Emergency Hospital for Children 'L. Turcanu' Timisoara between 2001 and 2006. Case distribution by etiology shows a clear prevalence of genetic determination: chromosomal 45 cases (24%), monogenic 17 cases (9%), and polygenic 86 cases (46%), in comparison with epigenetic etiology 39 cases (21%). The study of chromosomal anomalies case distribution with cardiac involvement shows an increased frequency of Down syndrome cases and cardiac disorders (50%). Cardiac pathology associated to some monogenic syndromes is obvious in Holt-Oram syndrome, Marfan syndrome, Bourneville tuberoses sclerosis, Hurler syndrome and Carpenter syndrome; the distribution is similar to the one reported in similar studies and correlated with the incidence of these diseases in general population.

**Conclusion:** Congenital cardiac diseases represent pathology difficult to quantify.

These patients and their families are confronted with dramatic situations because of diagnosis delay, absence of therapeutic response and, mostly, of lack of sanitary and social support.

#### P01.265

#### Preventive effect of periconceptional folic acid supplementation on the risk of congenital heart defects: A registry based case-control study in the Netherlands

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Evidence is emerging that multivitamins containing periconceptional folic acid supplementation protects against the occurrence of congenital heart defects (CHD). Postulating that folic acid is responsible for the reduction in CHD risk we used data from a large surveillance for birth defects (EUROCAT- Northern Netherlands registry from 1981 to 2006) to perform a case-control study to investigate the effect of periconceptional folic acid supplementation on CHD risk.

The cases consisted of mothers who delivered infants with isolated or complex heart defects, without any syndrome or genetic abnormality (N=613, years 1996-2005). The control group consisted of mothers who gave birth to children with a known chromosomal or genetic defect or infants with other congenital malformations (N=2385). In both the case and control group, mothers of children with oral cleft, urinary tract, limb reduction and neural tube defects were excluded, because the risk of these defects are probably reduced by maternal folic acid supplementation. Potential confounding factors of periconceptional folic acid use included; maternal body mass index, education, maternal age at delivery of index baby, smoking behaviour and alcohol use during pregnancy were explored.

Adequate use of periconceptional folic acid supplements revealed an odds ratio of 0.81 (95%CI 0.67-0.96) for all types of CHD. Subgroup analysis showed an odds ratio of 0.60 (95%CI 0.42-0.86) for isolated ventricular septal defects. Periconceptional folic acid supplements appear to reduce the prevalence of CHD with approximately 20%. Considering the relatively high prevalence of CHD worldwide the findings of this study are important for public health.

**P01.266****Clinical characterization and NIPBL mutation analysis of 42 Portuguese patients with Cornelia de Lange Syndrome**

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Cornelia de Lange Syndrome [CdLS (MIM#122470)] is a rare multi-system disorder (prevalence 1:10.000) characterized by psychomotor developmental and growth delay, distinctive facial dysmorphism, microcephaly and limb anomalies. Mutations in the NIPBL [5p13.1 (MIM\*608667)] gene that encodes for delangin, a protein involved in sister chromatid cohesion, have been described in 27-56% of patients. A smaller number of patients have been found to have mutations in two other genes SMC1A [Xp11.2 (MIM\*300040)] and SMC3 [10q25 (MIM\*606062)] thought to be involved in chromatin cohesion.

We present data on 42 Portuguese patients with Cornelia de Lange Syndrome, observed by Clinical Geneticists according to the same clinical protocol, including prenatal and birth history, development, physical features and multisystem involvement. According to their phenotype patients were classified as mild, moderate or severe. The entire coding region (exons 2 to 47) and exon-intron junctions of the NIPBL gene was sequenced. Population screening was carried out for undocumented variants. In mutation positive cases sequence analysis was extended to the parents. Ongoing sequencing of NIPBL to date revealed the presence of heterozygous mutations in 11 patients from 10 unrelated families, 7 of which are novel. Two individuals with a nonsense mutation in NIPBL are siblings with a mild phenotype, providing further evidence that CdLS familial cases may remain undiagnosed. We highlight the importance of a thorough clinical assessment in order to recognize milder phenotypes of CdLS. We discuss phenotype-genotype correlation and its implication in terms of clinical prognosis and the importance of molecular diagnosis for genetic counselling.

**P01.267****Clinical-epidemiological study of the congenital anomalies of the corpus callosum**

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The corpus callosum is the largest interhemispheric commissure of eutherian mammals. We've studied the Epidemiology of the Congenital Anomalies of the Corpus Callosum (CACC) throughout the ECEMC [Spanish Collaborative Study of Congenital Malformations] database. ECEMC is a hospital-based case-control study covering, in 2005, 22,96% of total of newborns in Spain. Here we present the results of the epidemiological study of some clinical and etiological characteristics of infants with CACC registered by ECEMC programme.

The ECEMC calculated a frequency of 1.08/10,000 births for the CACC. Non-specified type of agenesis was the most frequent group and total agenesis the most infrequent. Taking into account the clinical features, the isolated cases represented only 6% of total newborns; the syndromic ones accounted for 29%, and multiply malformed (several congenital defects without an identified etiologic link) represented 65%. If we correlate the clinical and the anatomopathologic types, the most frequent among the isolated cases was non-specified agenesis. Correlating the anatomopathology and the genetic aetiology, 48,21% of them had a chromosomal origin. It has been described an association of CACC with several chromosomal alterations: del(5p), del(1qter), del(18q), del(6p25), dup(3q) and del(10q).

In order to carry out a right follow-up of the cases with CACC, whether prenatal or postnatal, the most important points to take into account are: 1) detailed ultrasound of Central Nervous System, 2) precise familial anamnesis including three generations, 3) foetal or neonatal

high-resolution karyotype, and 4) Magnetic Resonance Imaging. All these facts will lead us to a genetic counselling in the best conditions.

**P01.268****New case of Cranio-Lenticulo-Sutural Dysplasia - a recently described genetic syndrome with late-closing fontanelles**

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We delineated Cranio-lenticulo-sutural dysplasia (CLSD; Boyadjiev-Jabs syndrome) as a new autosomal-recessive syndrome in a consanguineous family where five males and one female have similar craniofacial features (large and late-closing fontanelles, hypertelorism), early onset cataracts, and mild generalized skeletal dysplasia. Linkage analysis mapped the locus to chromosome 14q13-q21 and a F382L causative mutation was identified in SEC23A. Detailed molecular and biochemical analysis of wild type and mutant SEC23A, an integral member of the COPII-mediated ER-to-Golgi trafficking pathway, led to better characterization of intracellular trafficking in health and disease. A zebrafish morpholino model recapitulated the human phenotype. Recently, an unrelated individual with clinical features consistent with CLSD was identified. Molecular analysis of SEC23A identified a novel heterozygous SEC23A mutation involving a highly conserved residue. This missense mutation was inherited from the unaffected father and was not present in 400 control chromosomes. No mutations were found in the maternal alleles and SEC23A real-time PCR analysis showed normal expression of the alleles. Biochemical characterization by *in vitro* COPII budding assay is in progress. Our data suggest that CLSD may be more common than previously thought and should be considered in the evaluation of patients with late-closing fontanelles. Alternative inheritance patterns may exist for this syndrome.

**P01.269****A 340 kb de novo 16p13.3 microduplication encompassing only 5 genes**

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We describe an 8-year-old female who was shown by MLPA to have a *de novo* interstitial duplication of the Rubinstein-Taybi Syndrome region. Characterization by array CGH (Agilent) revealed a 340 kb duplication of 16p13.3 containing only 5 genes, one of them being CREBBP.

The patient was born at term after an uneventful pregnancy. Dysmorphic facial features and a left hip dysplasia, initially treated by cast and later by a Pavlik harness, were noted. Aged 19 months, global psychomotor delay was noted (sitting at 11 months, but no speech or walking), as well as dysmorphic facial features, with round face, erythematous cheeks, short hypoplastic nose, long convex philtrum, microstomia, and low-set small ears. Pelvis X-rays showed changes in the left femoral head, atypical for classical congenital hip dysplasia. Based on these findings, the hypothesis of some form of chondrodysplasia punctata was raised, but could not be substantiated radiologically. Follow-up confirmed the mild global learning disability with normal growth parameters, apart from a leg-length discrepancy, the left being 2 cm shorter.

The rarity of published patients with comparable duplications make genotype-phenotype correlations difficult. Given a previous report of a duplication 16p13 in a patient with chondrodysplasia punctata (Hunter, 1985), we hypothesise that overexpression of gene(s) in the microduplication is responsible for abnormal bone development leading to teratological hip dysplasia. Given the known role of CREBBP in skeletal and mental development, this gene represents an excellent candidate.

**P01.270****Genetic study of chromosome 5 aberrations****L. A. Hosny, S. Hammad, M. Shihab;****National Research Centre, Cairo, Egypt.**

This work presents clinical picture of 12 cases having chromosome 5 aberrations .

**Material & Methods:-** 6 children, a couple seeking premarital genetic counselling and 5 couples seeking advice for, intrauterine and neonatal deaths.Chromosomal study of the children, their parents and the couples was performed

**Results & discussion:** 5p- in 4 children diagnosed as cri du chat syndrome,three with normal karyotype of both parents . in the fourth child , the mother was a balanced translocation carrier t(5;22)(P14;P11.2) , sister was unbalanced carrier t(5;22)(P14;P11.2) , 5p+ and the aunt had 5p-. Fifth child had 46 , xy , del (5)P15.2 , inv (9)(P11;q12) showing features of both cri du chat and Goldenhar syndromes. The sixth child had 5p+ and his father was a balanced translocation carrier t(3;5)(P22;P15) . 5 paracentric inversion was found in the mother in a couple ,the other 5 couples showed chromosome 5 balanced translocation.

In one couple, the father was a balanced carrier t(5;7)(P15;P15) having a normal son with a balanced translocation and daughter with multiple congenital anomalies and a normal karyotype . The present study illustrates that chromosome 5 inversion or balanced translocation in one of the parents results in chromosome 5 aberrations in the offspring leading to intrauterine and neonatal deaths as well as genetic syndromes.

**CONCLUSION:-**Cytogenetic study of parents of children with chromosome 5 aberrations , couples seeking premarital genetic counseling or having repeated abortions, intrauterine and neonatal deaths is of considerable value to give a proper genetic counseling for next generations.

**P01.271****Expanding the mutational spectrum of CRLF1 in Crisponi Syndrome.****L. Crisponi<sup>1</sup>, A. Meloni<sup>1</sup>, F. Chiappe<sup>1,2</sup>, G. Zampino<sup>3</sup>, I. Okur<sup>4</sup>, S. Danda<sup>5</sup>, G. Crisponi<sup>6</sup>, F. Rutsch<sup>7</sup>;****<sup>1</sup>Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Monserrato, Italy, <sup>2</sup>Università degli Studi di Cagliari, Cagliari, Italy,****<sup>3</sup>Departments of Paediatrics, Catholic University, Rome, Italy, <sup>4</sup>Gazi University Medical School, Department of Pediatric Nutrition and Metabolism, Ankara, Turkey, <sup>5</sup>Clinical Genetics Unit, Department of Gastrointestinal Sciences, Christian Medical College, Vellore, India, <sup>6</sup>Casa di cura Sant' Anna, Cagliari, Italy,****<sup>7</sup>Department of General Pediatrics, University Children's Hospital, Muenster, Germany.**

Crisponi syndrome (CS) is a severe autosomal recessive disorder manifesting in infancy, characterized by contractions of facial muscles, dysmorphic features, camptodactyly, feeding and respiratory difficulties. Characteristic hyperthermic crisis frequently lead to death within the first months of life. Surviving patients usually develop a severe progressive kyphoscoliosis requiring corset therapy or corrective surgery. We have also observed paradoxical sweating after exposure to low ambient temperature in some affected adolescents. We found that mutations in the CRLF1 gene are associated with CS, showing allelism with Cold Induced Sweating syndrome type 1 (CISS1). We are currently expanding the mutational analysis of CRLF1 gene on more cases, which have been referred to our group. Up to now we found four different novel mutations; two missense mutations in an Italian patient, c.[338A>T;341T>C], p.[N113I;L114P]; one nonsense mutation in a Turkish patient, c.829C>T, p.R277X; the deletion of the entire exon 1 in an Indian patient. All these mutations are present on both alleles in the patients. CS and CISS1 belong to a group of conditions with overlapping phenotypes, also including Cold-Induced Sweating syndrome type 2 (CISS2) and Stüve-Wiedemann syndrome (SWS)/Schwartz-Jampel syndrome type 2 (SJS2), all caused by mutations of genes in the ciliary neurotrophic factor receptor (CNTFR) pathway. We are currently characterizing in details the clinical phenotype of all the patients collected with CS and CISS1 to establish a genotype/phenotype correlation. These studies may yield valuable information for prospective investigations of sweating disorders, thermoregulatory alterations and bone development.

**P01.272****Neonatal outcome of 937 children born after transfer of cryopreserved embryos obtained by ICSI and IVF and comparison with data of fresh cycles****F. Belva<sup>1</sup>, E. Van den Abbeel<sup>2</sup>, M. Camus<sup>2</sup>, P. Devroey<sup>2</sup>, J. Van der Elst<sup>2</sup>, I. Liebaers<sup>1</sup>, M. Bondue<sup>1</sup>;****<sup>1</sup>Medical Genetics, UZ Brussel, Brussel, Belgium, <sup>2</sup>Centre for Reproductive Medicine, UZ Brussel, Brussel, Belgium.**

**Background** To evaluate the safety of cryopreservation in combination with IVF or ICSI, prenatal diagnosis and neonatal outcome were investigated in children conceived from frozen-thawed ICSI embryos (cryo ICSI) and frozen-thawed IVF embryos (cryo IVF). Data were also compared with earlier published results from fresh ICSI and IVF embryos. **Methods** Questionnaire data and results of physical examination at 2 months of age of 547 cryo ICSI children and 390 cryo IVF children were also compared with those of infants born after transfer of fresh embryos.

**Results** Birth characteristics were comparable for cryo ICSI and cryo IVF. Cryo singletons showed a trend towards higher mean birthweight compared to fresh singletons, in ICSI and IVF, reaching significance when all cryo (ICSI plus IVF) singletons were considered. Low birth-weight rate according to multiplicity was comparable between fresh and cryo, in ICSI and IVF. Comparable rates of de-novo chromosomal anomalies (3.2%) were found in cryo ICSI fetuses/children versus the fresh ICSI group (1.7%) (Relative Risk 1.93; 95% CI 0.93-3.99). Major malformations were more frequently observed at birth in cryo ICSI liveborns (6.4%) than in cryo IVF liveborns (3.1%) (RR 2.08; 95% CI 1.09-3.95) and fresh ICSI liveborns (3.4%) (RR 1.89; 95% CI 1.30-2.76). **Conclusion** In cryo ICSI versus cryo IVF, prenatal and neonatal outcome results were comparable besides a higher major malformation rate in cryo ICSI. In the total cryo group versus the total fresh group, we found a higher mean birthweight in singletons and a higher major malformation rate in liveborns.

**P01.273****Autosomal Recessive Cutis Laxa Syndrome revisited****E. Morava<sup>1</sup>, U. Kornak<sup>2</sup>, D. Lefebvre<sup>1</sup>, Z. Urban<sup>3</sup>, S. Mundlos<sup>2</sup>, R. Wevers<sup>1</sup>;****<sup>1</sup>UMC Nijmegen, Nijmegen, The Netherlands, <sup>2</sup>Institute for Medical Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany, <sup>3</sup>Washington University, StLouis, MO, United States.**

The clinical spectrum of the autosomal recessive cutis laxa syndromes is highly heterogeneous, both with respect to organ involvement and severity. One of the major diagnostic criteria for cutis laxa is to detect abnormal elastin fibers in skin biopsy. In several other, clinically similar autosomal recessive syndromes, however, the classic histological anomalies are not present, and the clinical diagnosis remains uncertain. In some children with cutis laxa mutations have been demonstrated in the elastin and fibulin genes, but the underlying genetic etiology is still unknown in the majority of patients. Recently, mutations were discovered in the ATP6V020 gene in several consanguineous families with autosomal recessive cutis laxa. This genetic defect is associated with abnormal glycosylation in the Golgi apparatus, leading to a distinct combined N- and O-linked glycosylation disorder. Interestingly, similar mutations were also confirmed in patients with wrinkly skin syndrome, without the presence of severe skin symptoms with elastin deficiency. These findings suggest that the cutis laxa and wrinkly skin syndromes are phenotypic variants of the same disorder. The variable presence of protein glycosylation disorders in patients with diverse phenotype in the wrinkled skin-cutis laxa spectrum necessitates revisiting the definition of clinical diagnostic criteria in order to offer adequate prognosis assessment and counselling. Hereby we describe the spectrum of clinical features of the various forms of ARCL syndrome. Based on the recently unravelled novel disease entity we review the genetic aspects including genotype-phenotype relations and suggest a practical diagnostic approach.

**P01.274****Unusual malformative association in Dandy-Walker syndrome****C. Jurca, M. Bembea, C. Skrypnyk, K. Kozma, A. Jurca, S. Bembea, S. Costea;****Clinical Municipal Hospital "dr. G. Curteanu", Oradea, Romania.**

**INTRODUCTION.** Dandy-Walker syndrome (DWS) or Dandy-Walker complex (DWC) is a rare congenital anomaly (1:25000 live births)

characterized by vermis agenesis or hypoplasia, cystic dilation of the 4<sup>th</sup> ventricle and a large posterior fossa. The syndrome is defined by the presence of these three signs. There are three closely associated types of DWS: DWS malformation, DWS mega cisterna magna and DWS variant. OBJECTIVE. Presentation and discussion of three cases with different morphological and clinical forms of DWS. In all three cases, diagnosis was reached by incorporation of clinical (macrocephaly, seizures) and imaging (X-ray, CT, MRI) data. RESULTS. Patient #1 was diagnosed with Dandy-Walker malformation; he has a sister with Fraser syndrome. Patient #2 was diagnosed with a posterior fossa arachnoid cyst, left-sided Claude-Bernard-Horner syndrome, congenital heart disease (coarctation of the aorta, mitral stenosis) and gastosophageal reflux. Patient #3 was diagnosed with Dandy-Walker variant in a rare association with neurofibromatosis. CONCLUSIONS. DWS is a malformative association of the central nervous system with variable phenotype; it is frequently associated with other anomalies and an uncommon familial genetic load.

#### P01.275

##### De Morsier syndrome - case presentation

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Introduction: De Morsier syndrome is an extremely rare affection in medical practice, its diagnosis in the neonate period is exceptional. It is characterized by association of structural and functional anomalies of nervous system and endocrine glands (especially hypothalamus and hypophysis). The lesions of nervous system can involve optical nerves with optical nerve atrophy and agenesis of corpus callosum and cavum septum pellucidum.

Material and method: The presented patient is a girl with age of 6 month, female gender, held in our Clinics' evidence for prematurity, extreme hypotonia, agenesis of corpus callosum , nystagmus, eye disorders. The clinical - biological evolution was poor, with low level regarding stature and weight and major delay in psychomotor acquisitions.

Results: Several investigations and biological explorations were done: cranial ultrasonography - which shows agenesis of corpus callosum , stationary hydrocephaly ; MRI: agenesis of corpus callosum, increased ventricles dimensions.

Oftalmological examination and eye ultrasound shows optical nerve atrophy. The presence of the early puberty signs (pubic hair ) and statural deficit are signs of endocrine disorders without being major modifications of hormonal doses.

Conclusions: Extremely rare affection in pediatric medical practice. Positive diagnosis is established based on association of major clinical signs: eye manifestations (optical atrophy, nystagmus) early puberty, agenesis of corpus callosum , confirmed by specific investigations: cranial ultrasonography , MRI, specific blood tests..

#### P01.276

##### F142L mutation in GJB2 gene in a patient with uncommon skin disorder and deafness

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Dominant mutations in the human GJB2 gene, which encodes connexin 26 (Cx26) can cause non-syndromic hearing loss, but can also manifest with various skin disorders including palmoplantar keratosis (PPK), Vohwinkel syndrome, Bart-Pumpfrey syndrome (BPS) and keratitis-ichthyosis-deafness (KID) syndrome. We present a girl with congenital hearing impairment, plantar keratosis, extensive skin changes in form of follicular inflammatory papules, and erythematous, often scaly patches affecting whole body, including scalp and face. In addition, she had extensive mucosal involvement including oral and esophageal mucosa and perigenital region. Her hair was sparse and thin, she had submucosal cleft palate, and hypodontia. No other abnormalities were observed. Laboratory studies excluded immune/ autoimmune deficiencies. Karyotype and FISH for 22q11.2 microdeletion were normal. Sequencing of the coding region of the GJB2 gene revealed a de novo heterozygous F142L mutation located in the third transmembrane domain of the Cx26 gene. This mutation has been

reported only once, also in a patient with unusual mucocutaneous findings and deafness. Our patient confirms the pathogenic nature of this mutation, delineating associated clinical manifestations. It also points out at the broad and overlapping nature of ectoderm derived tissue changes due to the autosomal dominant GJB2 mutations.

#### P01.277

##### X-linked deafness type 3 (DFN3) phenotype associated with a paracentric inversion

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We report a 7 year old male with severe sensorineural hearing loss diagnosed at 8 months. His features included a round face, hypertelorism, epicanthic folds and a broad nasal root. Initial developmental concerns resolved once he was in an appropriate educational program. Sequencing for GJB2 and GJB6, Fragile X testing, echogram, and abdominal ultrasound were normal. An ECG revealed an incomplete RBBB. The CT scan revealed a large fundus of the internal auditory canals and absence of the bony partition between the fundus and the adjacent cochlear turns with a widened modiolus bilaterally. Therefore, he was at risk for a fistulous communication between the subarachnoid space and inner ear resulting in a perilymphatic gusher upon stapes manipulation. These findings are consistent with those described in persons with DFN3 hereditary deafness. His karyotype was 46,inv(X)(q13q24),Y.ish inv(X)(XIST+). Successive FISH experiments refined the breakpoints to inv(X)(q21.1q22.3). The Xq21.1 breakpoint was narrowed to a 25 kb region about 450 kb centromeric to the DFN3 gene, POU3F4. Other DFN3 patients lacking mutations within POU3F4 have been reported. Ten had deletions centromeric to POU3F4, one had an inversion and deletion centromeric to POU3F4 and one had a duplication centromeric to POU3F4 and an inversion including POU3F4. In the present case there were no detectable deletions or duplications near the Xq21.1 breakpoint. Thus, we hypothesize that the hearing loss phenotype in this patient is caused by dysregulation of POU3F4 due to separation from *cis*-acting regulatory elements. The patient's asymptomatic mother had a karyotype of 46,X,inv(X)(q13q24)[19]/45,X[11].

#### P01.278

##### Polymorphisms in the glucocorticoid receptor in children with difficult bronchial asthma

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Objective. We investigated allele and genotype frequencies of the BclI and Tth111I polymorphisms in the glucocorticoid receptor (GR) gene among children with difficult bronchial asthma.

Patients and methods. Our study group consisted of 59 children (43 boys and 16 girls) in age of 2-17 suffering from difficult asthma and control group consisted of 151 healthy children (78 boys and 73 girls) in age of 4-17. The BclI and Tth111I polymorphisms were detected by PCR-RFLP using the primers and methods previously described (Fleury I. et al., 2003; Van Rossum EFC. et al., 2004). Data were compared through Chi-square test.

Results. Table 1. Genotype frequencies in asthma patients and controls

Genotypes	Study group (59)		Control group (151)	
	boys 43 (72,9%)	girls 16 (27,1%)	boys 78 (51,7%)	girls 73 (48,3%)
BclI-CC	39,5 %	37,5 %	32%	34,2%
BclI-CG	48,8 %	50 %	51,3%	52,1%
BclI-GG	11,6 %	12,5 %	16,7%	13,7%
Tth111I-CC	36,6 %	37,5 %	47,4%	49,3%
Tth111I-CT	56 %	50 %	43,6%	39,7%
Tth111I-TT	7,3 %	12,5 %	9,0%	11,0%

Conclusion. Allele and genotype distributions of BclI and Tth111I polymorphisms of the GR gene were similar in asthma patients and controls and previously reported populations. There were no significant differences in allele and genotype frequencies in groups of children with different sex.

**P01.279****Dilated cardiomyopathy with hearing loss - a new form?**

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**Background:** Dilated cardiomyopathy (DCM) is a myocardial disorder characterized by ventricular dilatation, and impaired systolic function leading to heart failure and death. To date 12 genes and 10 chromosomal loci have been associated with this condition, but only autosomal-dominant DCM, 1J (MIM: #605362) caused by mutations in *EYA4* gene is also associated with sensorineural hearing loss (SNHL).

**Methods:** Clinical examination of parents and four children was performed, including ECG, Holter, Echo-CG; blood and urine biochemistry, and tandem mass-spectrometry (to exclude mitochondrial diseases). The phenotype of a deceased sibling was obtained from hospital and out-patient information. Genetic testing was performed by direct sequencing of coding and adjacent intronic areas of the *EYA4* gene.

**Results:** An Uygur family with DCM was investigated. The proband and the oldest brother had progressive SNHL, complete A-V block, DCM, and died due to heart failure at the age of 14 y.o. and 9 y.o., respectively. Two younger brothers had incomplete A-V block, LBBB, and SNHL at the age 6 y.o. and 11 y.o. All symptoms became more pronounced with age. A sister (9 y. o.) has normal hearing but A-B block(I) and incomplete LBBB. Both parents are healthy. We didn't find any mutations of *EYA4* gene. Unfortunately, the small size of the family and unclear phenotype of the 9 y.o. girl precludes informative linkage analysis. Pedigree analysis reveals recessive inheritance of DCM, conduction defects and SNHL (autosomal or X-linked) in this Uygur family. We propose that this family has a novel genetic form of DCM.

**P01.280****Novel Tyrosine Hydroxylase Gene Mutation In Three Turkish Siblings With Dopamine-Responsive Dystonia**

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Dopa-responsive dystonia (DRD) is a rare, autosomal dominant (GTP-cyclohydroxylase gene mutation) or rarely recessive (tyrosine hydroxylase gene mutation) inherited disorder. Both enzymes take part in dopamine synthesis. Their deficiencies cause the dopamine level reduction. The first clinical symptoms occur in the childhood

We report here three siblings who were borned to first cousins; with a novel recessive mutation in Tyrosine hydroxylase gene that results in dopa- responsive dystonia. Older brothers were monozygotic twins. They are now at the age of 4<sup>6/12</sup> and 1<sup>9/12</sup> years. All of them have development delay and also motor dysfunction. They displayed extrapyramidal signs in early infancy. The clinical diagnoses were confirmed by mutation analyses of Tyrosine hydroxylase gene which detected a novel mutation of P492R (1475 C>G) in the homozygotic state. After diagnosis, L-DOPA treatment was started, however clinical picture did not change. Therefore selegiline (selective MAO-B inhibitor) was added to therapy. Low dose L-DOPA and selegiline markedly improved clinical picture.

Here we are presenting the clinical features and outcomes of the L-DOPA/Selegiline treatment in three siblings with Dopa-responsive dystonia results from a novel recessive mutation in Tyrosine hydroxylase gene, reviewing of the literature.

**P01.281****Familial duplication 8p without phenotypic effect**

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We report a family where 3 members have rearrangement of chromosome 8 with normal phenotype. A proband 14-year-old girl was tested because of hypogonadism. G-banded chromosome studies were carried out and showed karyotype 46, XX, der (8). Her father's karyotype was normal - 46, XY, but mother and 28-year-old sister had the same chromosome 8 rearrangement without any clinical signs. The elder sister's 3-year-old daughter had normal phenotype.

Later at the age of 18 the proband was tested again during pregnancy. An abnormal chromosome 8 with additional material of unknown origin on the terminal region of short arm was tested. To identify this finding FISH analysis was performed using painting (wcp) and telomere spe-

cific (tel) DNA-probes for chromosome 8. FISH characterization of the abnormal chromosome with wcp 8 and tel 8p probes was estimated as duplication of a short-arm segment 8p23: 46,XX, add(8)(p23). ish dup(8)(p23)(wcp8+,pter+) (ISCN 2005). Euchromatic abnormality of no phenotypic consequence such as euchromatic duplication in terminal region p23.1-p23.3 of chromosome 8 is known as chromosome variant. Variant chromosomes being normal chromosomes behave normally at meiosis and show 1:1 segregation. A person carrying a variant chromosome has no increased risk for having abnormal offspring. Normal results of maternal serum biochemical screening and high quality ultrasound scanning during the pregnancy had allowed to avoid an invasive procedure. After 40 weeks of pregnancy there was born male newborn without any clinical signs of chromosomal pathology.

**P01.282****DYSCKERNE: An electronic Dysmorphology Diagnostic System (DDS)**

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Over 2,500 rare and difficult to diagnose conditions presenting with patterns of birth defects have been identified. The rarity of these dysmorphic conditions means that even in Centres of Expertise, experience may be limited resulting in delayed or uncertain diagnosis. Making a 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.

One of the main aims of the DYSCERNE project ([www.dyscerne.org](http://www.dyscerne.org)) is to develop an electronic Dysmorphology Diagnostic System (DDS) which will link existing European Centres of Expertise to form a powerful diagnostic resource for rare dysmorphic conditions.

The DDS will allow clinicians to submit difficult to diagnose cases, for evaluation by an international panel of experts. A diagnostic report including suggested management plans for the patient will be prepared from the consensus of expert opinions and sent to the submitting clinician. Case histories will be stored in an archive which will be reviewed periodically.

An on-line educational tool for the description of dysmorphic features will also be developed which will increase diagnostic skills in the evaluation of rare dysmorphic diseases.

The DDS will facilitate rapid and equitable access for clinicians from all EU countries to expert opinions. It will increase capacity and accuracy of diagnoses and decrease time from presentation to diagnosis. This will facilitate definition and classification of rare dysmorphic conditions and promote further clinical research into these complex disorders.

**P01.283****Clinical Variability in Acro-(Cardio)-Facial-Syndrome**

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In 1987 Richieri-Costa and Orquiza described a Brazilian patient born to consanguineous parents with ectrodactyly, cleft lip/palate and congenital heart defect. Four additional cases with ectrodactyly, genital anomalies, congenital heart defect and cleft lip/palate or high arched palate have been published [Giannotti et al., 1995; Guion-Almeida et al., 2000; and Mingarelli et al., 2005].

Giannotti et al. [1995] reported on a brother and sister with cleft palate, cardiac defect, genital anomalies and ectrodactyly, suggesting the acronym CCGE standing for cleft palate, cardiac defect, genital anomalies and ectrodactyly. An autosomal recessive pattern of inheritance was suggested considering these pedigrees. Guion-Almeida reported on a 4-month-old infant with ectrodactyly, clefting ear anomaly, CHD, cortical atrophy of the brain and growth retardation, with possible acro-cardio-facial Syndrome (ACFS).

We report on a 25-year-old man with ectrodactyly and genital anom-

lies whose parents are first cousins. Their second child died 4 days after birth with severe limb defects and imperforate anus. Our patient may represent clinical variability of the acro-cardio-facial syndrome. If our case has the ACF syndrome, it would be the mildest form of this condition.

#### P01.284

#### **Unusual pattern of inheritance and orodental changes in the Ellis-van Creveld Syndrome**

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Ellis-van Creveld (EVC) syndrome (chondroectodermal dysplasia, mesoectodermal dysplasia, OMIM 225500) is an autosomal recessive skeletal dysplasia characterized by short limbs, short ribs, postaxial polydactyly and dysplastic nails and teeth. Oral manifestations tend to be pathognomonic such as multiple broad labial frenula and congenital missing teeth.

In this study we report 3 Egyptian families with six cases of EVC syndrome.

An unusual pattern of inheritance with father to son or to daughter transmission was observed in 2 consanguineous families thus demonstrating quasidominant inheritance, probably for the first time in the literature. A new consistent orodental anomaly found in all our cases was bifid tip of the tongue. We emphasize study of orodental anomalies in future cases for accurate diagnosis of Ellis-van Creveld syndrome and its probable differential diagnosis from Weyers Acrodental dysostosis.

#### P01.285

#### **Encephalocraniocutaneous lipomatosis: A propos of a boy with an unusual pattern of genital anomalies**

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Encephalocraniocutaneous lipomatosis (ECCL) is a sporadic, congenital neurocutaneous disorder, characterized by cerebral, ocular and cutaneous abnormalities, including asymmetrical cerebral atrophy, intracranial and spinal lipomas, mental retardation and/or epilepsy, epibulbar dermoids or choristomas, alopecia, facial skin-tags, craniofacial lipomas and a peculiar hairless fatty tissue nevus of the scalp, designated as psiloparius. The pathogenesis remains undetermined; one hypothesis is a lethal autosomal dominant mutation only surviving in a mosaic state. There is a considerable overlap with other neuroectodermal disorders, as oculocutaneous syndrome (OCCS), Goldenhar syndrome, and epidermal nevus syndrome. Recently, some clinical features of ECCL and OCCS were reviewed and diagnostic criteria were established. Herein, we describe a male infant whose clinical signs suggest ECCL. Besides the main features, he also had a compound odontoma, already reported but not frequent in ECCL and in similar conditions. However, the pattern of genital anomalies has not been reported so far. It includes asymmetrical penoscrotal transposition, ectopic left hypoplastic hemiscrotum, and a pedunculated perineal mass, whose aspect suggested a rudimentary accessory scrotum; the phallus had a normal length with an increase of subcutaneous tissue in prepuce region. This phenotype is quite rare and has been described in association with perineal lipoma or lipoblastoma, which probably arose in the perineum and divided the moving labioscrotal swelling into three parts, during early fetal life. The same mechanism is proposed in present case, considering that in ECCL there are a few reports of lipomas, lipomatous mass and/or skin-tags located outside the craniofacial region, including in genital area.

#### P01.286

#### **„Stat rosa pristina nomine, nomina nuda tenemus“: a survey on attitude of Italian clinical geneticists towards eponyms**

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An eponym is a person after whom a discovery, invention, etc., is named. Eponyms are widely used in all clinical disciplines. Recently, a provocative editorial published in British Medical Journal argued that their use should be abandoned. The London Dysmorphology Data-

base currently reports >5000 diseases: 44% of them are designated by eponyms.

We wondered how eponyms are perceived among clinical geneticists in Italy. By administering a questionnaire on the use of eponyms, we wanted to explore also the attitude of clinical geneticists towards nomenclature and its regulation. The home-made, Likert-type questionnaire consisted of 10 items exploring the attitude towards eponyms through the following domains: role of historical aspects, value in learning, use in clinical practice, facilitation of scientific discussion, convenience. Two additional items pertained to the need for rules in nomenclature. Scores were modelled as discrete variables and analysed by descriptive statistics.

We collected 102 (84%) fully filled-in questionnaires. The median value of the total score modelling the attitude towards eponyms was close to neutrality, with a trend in favour of eponyms. When the participants were asked to state a radical position (keep or abolish), 73% answered to prefer keeping use of eponyms. We found a marginally significant correlation between attitude in favour of eponyms and both age and years of practice. Regardless their position with respect to eponyms, the vast majority stated that the use of nomenclatures should be ruled by guidelines. Our findings provided a surprising impression of interest in the subject and underscored the need for recommendations.

#### P01.287

#### **Molecular diagnosis of familial mediterranean fever in Armenians**

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Familial Mediterranean Fever (FMF) is an inherited, recessively transmitted inflammatory condition usually occurred in populations from Mediterranean decent. The prevalence of heterozygous carriers of one of the mutations of MEFV gene is as high as 1 in 5 healthy individuals in Armenians.

Genetic testing of this rare Mendelian disorder (MIM no 249100) is efficient for early diagnosis, especially for atypic cases. Certain mutations have significant correlation with renal amyloidosis, the most severe possible manifestation of FMF. Also genetic testing is very important for colchicine therapy correction.

Twelve MEFV mutations are identified in more than 8000 FMF patients (heterozygotes, homozygotes and compound heterozygotes) in comparison with healthy individuals has revealed the most frequent mutations and genotypes. Every week we have 35-50 new cases. We have revealed that FMF is caused by presence of single mutation in 18.6% of heterozygote carriers.

Our results confirm that the MEFV gene analysis provides the objective diagnostic criterion for FMF (characterisation of the two MEFV mutated alleles in more than 90% of the patients). Molecular testing is also used to screen the MEFV gene for mutations in patients with a clinical suspicion of FMF. We also demonstrated the unfavourable prognostic value of the M694V homozygous genotype, and provided the first molecular evidence for incomplete penetrance and pseudodominant transmission of the disease. Overall, these data, which confirm the involvement of the MEFV gene in the development of FMF, should be essential in clinical practice, leading to new ways of management and treatment of FMF patients.

#### P01.288

#### **Midline Facial Defects with Hypertelorism: investigation of neuropsychological aspects and 22q11.2 deletion by FISH**

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Midline facial defects with hypertelorism (MFDH) are a group of rare and heterogeneous condition involving anomalies of frontonasal process. In some patients it is associated with structural and functional anomalies of the central nervous system. These CNS abnormalities have similarity with those found in patients with 22q11.2 deletion syndromes. In addition, there are some isolated reports of MFDH in which 22q11.2 deletion were detected. Furthermore, even in the absence of anatomical abnormalities detected by neuroimaging, functional disabilities could be present in patients with MFDH, which would be better investigated by neuropsychological assessment. Therefore, the main

objectives of our study were to characterize the neuropsychological aspects of patients with MFDH, to correlate them with neuroimaging findings and to investigate the 22q11.2 deletion in these patients. Neuropsychological evaluation was performed using the Luria Nebraska Battery and WISC for children and WAIS for adults; the 22q11.2 deletion was investigated by FISH. Heterogeneous results on neuropsychological evaluation involved difficulties cognitive domains such as picture arrangement, expressive language, comprehension, arithmetic, digit span and motors ability. These abnormalities are similar, in part, to those reported in deletion of 22q11.2 region, which was not detected in all cases. In conclusion, we found a relationship between neuropsychological and radiological CNS alterations in MFDH. As the 22q11.2 region is recognized as critical for embryonal development of midline, in view of the clinical-genetic heterogeneity and the specificity of the probe used, the involvement of 22q11.2 region in the etiology of MFDH needs to be further investigated.

#### P01.289

#### Associated malformations in patients with gastroschisis and omphalocele

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The etiology of gastroschisis and omphalocele (exomphalos) is unclear and their pathogenesis is controversial. However, the reported types and frequency of malformations associated with omphalocele and gastroschisis vary between studies. The purpose of this investigation was to assess, in a geographically defined population, the prevalences at birth of associated malformations in patients with omphalocele and gastroschisis which were ascertained between 1979 and 2003 in 334,262 consecutive births. Of the 86 patients with omphalocele, 64 (74.4%) had associated malformations including chromosomal abnormalities (25 cases, 29.0%); non chromosomal recognized syndromes including Beckwith-Wiedemann, Goltz, Marshall-Smith, Meckel-Gruber, Oto-palato-digital type II, CHARGE, and fetal valproate; sequences, including ectopia cordis, body stalk anomaly, exstrophy of bladder, exstrophy of cloaca, and OEIS; malformation complex including Pentalogy of Cantrell, and patients with non syndromic multiple congenital anomalies (MCA) (26 cases, 30.2%). Malformations of the musculoskeletal system (23.5%), the urogenital system (20.4%), the cardiovascular system (15.1%), and the central nervous system (9.1%), were the most common other congenital anomalies occurring in patients with omphalocele and MCA. For gastroschisis, the total prevalence was 1.79 per 10,000. However, there was a significant increase over the study period in the total prevalence. The maternal age-specific prevalence was highest in the 15-19 year age group. Of the 60 patients with gastroschisis, 10 (16.6%) had associated malformations including one skeletal dysplasia, one amyoplasia congenita, and 7 non syndromic MCA. In conclusion the overall prevalences of malformations associated with omphalocele and gastroschisis are quite different and emphasizes the need for a thorough screening of cases for other malformations.

#### P01.290

#### Discordance of primary congenital glaucoma in monozygotic twins

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Glaucoma is a heterogeneous group of optic neuropathies characterized by degeneration of the optic nerve, usually associated with elevated intraocular pressure. It is the cause of 15% of blindness worldwide. Primary Congenital Glaucoma (PCG), one of the three major forms of the disease, becomes apparent at birth or before the age of three and is a major cause of childhood blindness. Mutations in both alleles of the cytochrome P450B1 (CYP1B1) gene, which is the only gene thus far linked to PCG, result in the disease phenotype. It has been recently shown that mutations in this gene are cause of disease in approxi-

mately 70% of Iranian PCG patients and that the common mutations in the population are G61E, R368H, R390H, and R469W.

We report here the observation of highly variable expression of primary congenital glaucoma in two individuals who are identical twins. Both carried the G61E mutation in the homozygous state. The identical twin status of the individuals was confirmed using several microsatellite markers.

#### P01.291

#### Cloning & Expression of Human rFVII in Insect Cells

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Hemophilia is the one of the most prevalent genetic disorders. It is inherited as sex-linked pattern. Obtaining factor from plasma or by recombinant technology, in high dose is one of the major challenges for treatment of Hemophilia. However 25% of these patients naturally raised antibody against factor VIII. Viral contamination and availability in very low dose is another challenge to obtain factor VIII from plasma. Administration of Recombinant factor VII for patients who raised antibody against FVIII can be one of the solution for mention problem. Isolation, cloning and expression of recombinant FVII by Gateway technology was the aim of this study. Factor VII gene was isolated from HepG<sub>2</sub> cell line and cloned to TOPO vector by TOPO cloning method. The construct was ligated to Baculovirus destination vector by LR recombination using gateway technology and the recombinant virus was transfected to, insect cell line, SF9. Expression of recombinant FVII was detected by SDS-PAGE, ELISA and western blot analysis.

#### P01.292

#### A prenatally detected case of congenital hepatoblastoma

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Hepatoblastoma is a rare tumor of childhood. The incidence of hepatoblastoma in the first year of life is about one in a million. The mean time of its onset is 14 to 24 months. Forty-two congenital hepatoblastoma cases were reported so far. Among 42 congenital hepatoblastoma patients, seven cases have been detected in the prenatal period. Only one out of seven cases detected in the prenatal period has been diagnosed as hepatoblastoma. The etiology of hepatoblastoma is unknown. However, it has been shown to be associated with prematurity, low birth weight, hepatitis B, familial adenomatous polyposis, Beckwith-Wiedemann syndrome and hemihypertrophy. In this report, we report a rare case of hepatoblastoma detected before birth and confirmed by postmortem.

#### P01.293

#### Genotype-phenotype correlation in hereditary hemorrhagic telangiectasia in patients with ACVRL1 mutations: Is c.1112dupG mutation a milder mutation?

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**Introduction:** Hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder characterized by recurrent epistaxis, cutaneous telangiectasia, and visceral arteriovenous malformations (AVMs) that affecting lungs (PAVM), gastrointestinal tract (DAVM), liver (HAVM), and brain (CAVM) and resulting from mutations in two major genes: ENG (HHT1) or ACVRL1 (HHT2).

Our objective was to determine the influence of c.1112dupG mutation on clinical phenotype in HHT2 patients.

**Methods:** We retrospectively compared the frequency of clinical features of HHT between a subgroup of patients with mutation c.1112dupG (group A) and those with the other ACVRL1 mutations (group B), using

a clinical HHT database (CIROCO), build by the HHT reference centre in France and used by the French-Italian Network.

**Results:** 351 HHT2 patients were included in 6 HHT centres in France. Eighty three patients were in group A and 268 patients were in group B. Epistaxis were present in both groups and occurred at a younger age in group B patients (Median = 16 vs 21 years old, p<0.001). Pulmonary involvement appeared to be significantly more frequent in group B patients (8 vs 18 %, p=0.03). Frequency of hepatic, digestive and neurological AVM were not significantly different in both group but hepatic AVM were more severe in group B. Indeed, no hepatic transplantation were performed in group A (0 versus 5%, p= 0.04).

**Conclusion:** This study pointed out major differences between clinical expression of *ACVRL1* mutation c.1112dupG, which appeared to be milder, than other *ACVRL1* mutation phenotypes.

#### P01.294

##### Holoprosencephaly mutations in the Dutch population

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Holoprosencephaly (HPE) is a common severe malformation of the brain that involves abnormal formation and septation of the developing central nervous system. The prevalence is 1:250 during early embryogenesis, but the live born prevalence is only 1:16000. The etiology of HPE is extremely heterogeneous and can include both a teratogenic and/or genetic basis. We studied four genes known to be involved in HPE, namely *SHH*, *ZIC2*, *SIX3* and *TGIF* by sequence and MLPA analysis. A series of in total 72 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 17 pathogenic mutations (24%) in total, of which 2 in *SHH*, 6 in *ZIC2* and 9 in *SIX3*. Only one mutation (Alanine-tract expansion in *ZIC2*) was reported previously and detected twice in this population, all others were novel. Two mutations were complete gene deletions (one *SIX3*, one *ZIC2* deletion) of which the deletion sizes were further characterized using the 250K *nsp* I Affymetrix SNP array. The familial mutations displayed great phenotypic heterogeneity of the disease, which makes it difficult to establish genotype-phenotype correlations. This phenotypic variability may be due both to environmental factors and to potential modifier genes. HPE development is probably a multihit process, which implicates more genes; illustrating the importance of further identification of new genes.

#### P01.295

##### Huntington disease and Huntington-like phenotype: 10 years of local molecular diagnostic experience

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Only 58% of all diagnostic requests were confirmed; from those excluded, we selected 200 patients and studied them for HD-Like genes: an eight extra octapeptide repeats in the *PRNP* gene (HDL1); a CTG/CAG repeat in the junctophilin (*JPH3*) gene (HDL2); a CAG expansion in two SCA genes - *ATN1* (DRPLA) and *TBP* (HDL4/SCA17); as well as other included in the differential diagnosis of HD: neuroferritinopathy (*FTL* gene) and benign hereditary chorea (*TITF1* mutations).

Expansion of CAG repeats in *ATN1* and the insertion on *PRNP* were excluded in all cases. One family (mother and son with chorea since childhood, myoclonus, falls and dysarthria) carried a nonsense mutation in *TITF1*. A *FTL* mutation was detected in one Gypsy family (mother asymptomatic and son with mild non-progressive mental retardation, and gait disturbances by age 13; both had with pallidal involvement on MRI). We found also a CAG expansion in *TBP* (a patient with behavioural disturbances, epilepsy, aphasia, imbalance, and gait ataxia). Finally, we found a 47 CTG/CAG expansion in the *JPH3* gene, in a Brazilian patient (onset at age 44 years of bradipsychism, mutism, dysarthria, cognitive deterioration and chorea, as well as ataxic gait;

he had cortical atrophy).

Study of HD-like disorders is warranted, whenever HD has been excluded and it is clinically indicated.

#### P01.296

##### Lifestyle factors and the age at onset of Huntington disease

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Huntington disease (HD) is an autosomal dominant neurodegenerative disorder due to an expanded CAG repeat in the *IT15* gene. Transgenic HD mouse model studies have shown that raising mice in an enriched environment delays the onset of symptoms, leading us to consider whether pre-morbid lifestyle affects age-at-onset in humans. Subjects with symptomatic HD were interviewed using a questionnaire to ascertain pre-morbid lifestyle during three life stages (teens, 20s/30s, 40s/50s). Recorded activities were classified as physical, intellectual or passive, and activity scores generated. Surveys were matched with the subject's age-at-onset and CAG repeat length.

Preliminary analysis (n=92) showed a mean age-at-onset of 44.9 years (range 21-76), with a strong inverse correlation to CAG repeat length ( $r=-0.728$ ,  $p<0.001$ ). Linear regression indicated a negative association between average pre-morbid leisure-time passivity and age-at-onset ( $b=-0.744$ ,  $R^2=0.109$ ,  $p=0.001$ ) that remained significant when adjusted for CAG repeat length ( $b=-0.333$ ,  $R^2=0.55$ ,  $p=0.048$ ). This association was most apparent for passivity during the teens and in men. Comparison of the mean age-at-onset in groups below and above the median passivity score showed a difference of 6.0 years (95%CI=1.2 to 10.7). No significant relationship was demonstrated between average intellectual or average physical leisure-time activity and age-at-onset or CAG repeat length. Data from over 150 interviews in Australia and New Zealand will be presented.

Passivity in leisure-time is associated with age-at-onset of HD, and CAG repeat length, suggesting that passivity contributes to earlier onset of symptoms, or is a preclinical manifestation of HD, more apparent in those with larger CAG repeat lengths.

#### P01.297

##### Familial autosomal dominant hyperinsulinism due to *SUR1* (*ABCC8*) mutation

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Familial congenital hyperinsulinism is responsible of prolonged hypoglycemia, secondary to mutations of genes involved in the regulation pathway of insulin secretion by  $\beta$  Langerhans cells. Most of the cases are in relation with recessive mutations of two genes, located in 11p15.1, named *SUR1* (*ABCC8*) and *KIR6.2* (*KCNJ11*), and coding for two subunits of the same potassium channel.

Dominant mutations of *SUR1* (*ABCC8*) were only published in two families reported by Thornton (1998 and 2003) and Huopio (2000 and 2003). In these families, the hypoglycaemia was sensible to diazoxide. In the family reported by Huopio, the eventuality of transformation in diabetes with age was noticed.

We report the observation of a female newborn presenting with severe hypoglycaemia at 36 hours of life, requiring continuous gastric nutrition at the beginning, but compatible with a normal breath feeding when diazoxide was started. Her father had similar symptoms in childhood, improved by diazoxide. In the two patients, a missense heterozygous mutation of *SUR1* (*ABCC8*) was discovered (2143G>A - V715M). Screening of other genes involved in familial dominant hyperinsulinism was negative (*HNF4*, *KIR6.2*). The mutation was not present in the two paternal grand-parents of the index case.

**P01.298****Hyperphosphatasia with seizures, neurologic deficit and characteristic facial features: Five new cases of Mabry syndrome**

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In 1970, Mabry et al. described multiple cases of persistent hyperphosphatasia associated with developmental delay and seizures in a single consanguineous family (OMIM#239300). The nosology of this condition, however, is uncertain. We report five new cases that help delineate this disorder and provide further evidence favouring autosomal recessive inheritance, with sib recurrence in one instance (French non-consanguineous parents), and consanguinity (Lebanese parents) in another. Common to all five children is the Mabry triad of tonic-clonic seizures (usually beginning around one year of age), moderate to severe developmental delay, and persistently elevated alkaline phosphatase activity, without any indication of liver or metabolic bone disease. The degree of hyperphosphatasia varies considerably amongst cases (~1.3 to 20 times the upper age-adjusted reference limit). In addition, all five display a common facial dysmorphism, characterized by hypertelorism, broad nasal bridge, and tented mouth. The three singleton cases also have brachytelephalangy, but there is no evidence of skeletal anomalies in the two siblings. In the family first described by Mabry et al., at least one affected was noted to have intracellular inclusions on biopsy of rectal mucosa but not liver. In three of our cases, inclusions have been observed in cultured cells, but the cells are not uniformly perturbed and characterization is still in progress. We are aware of additional cases in the literature which suggest a wider phenotypic spectrum, but they are consistent with an autosomal recessive condition characterized by hyperphosphatasia, seizures, and neurologic deficit - a disorder we call Mabry syndrome.

**P01.299****The yield of cascade screening and risk stratification for sudden cardiac death in hypertrophic cardiomyopathy MYBPC3 gene mutation carriers**

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**Background:** Hypertrophic cardiomyopathy (HCM) is a common autosomal dominant heart disease associated with heart failure and sudden cardiac death (SCD). Disease penetrance and the risk of SCD in mutation carriers are unknown. We investigated the prevalence of a clinical diagnosis of HCM and the presence of risk factors for SCD at the first cardiological evaluation after presymptomatic genetic testing in asymptomatic carriers of a MYBPC3 gene mutation.

**Methods:** 235 asymptomatic mutation carriers were cardiologically evaluated on the presence of HCM and risk factors for SCD. A comparison was made for different types of MYBPC3 gene mutations.

**Results:** A clinical diagnosis of HCM could be made in 22.6% of carriers. Disease penetrance at 65 years was incomplete for all types of MYBPC3 gene mutations. Women were affected less often ( $p=0.003$ ) and disease penetrance was lower ( $p=0.024$ ). 22 asymptomatic carriers had  $\geq$  two risk factors for SCD. In nine a clinical diagnosis of HCM could be made and they were therefore at high risk for SCD.

**Conclusion:** A diagnosis of HCM can be made in almost one quarter of mutation carriers at first evaluation. Disease penetrance of HCM in MYBPC3 gene mutation carriers is incomplete at 65 years and differs between men and women. Risk factors were frequently present and 4% of carriers appeared to be at high risk for SCD. Our data justify presymptomatic genetic testing in HCM families with a pathogenic mutation and frequent cardiological evaluation on the presence of HCM and risk factors for SCD, even until advanced age.

**P01.300****Prevalence and phenotypic characteristics associated with MyBPC3 mutations in patients with hypertrophic cardiomyopathy**

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**Background:** Mutations in the beta-myosin heavy chain (MYH7) and myosin-binding protein C (MyBPC3) genes are the most frequent causes of hypertrophic cardiomyopathy (HCM). MyBPC3 mutations have been associated with later diagnosis and less hypertrophy than MYH7 mutations. Our objectives were to compare the prevalence of mutations and the phenotypic characteristics associated with both genes in our patients with HCM.

**Methods:** SSCP analysis and sequencing of fragments with abnormal MyBPC3 gene mobility were carried out in 130 consecutive index patients with HCM previously studied for the MYH7 gene (10% mutated). The phenotypes of patients with and without mutations in both genes were compared.

**Results:** We identified 16 MyBPC3 different mutations (8 of them novel) in 20 patients (15.4%). Age at diagnosis was similar in MyBPC3 vs. MYH7 patients (46.2 vs. 46.0, p ns). More than 50% (11) of MyBPC3 mutated patients were diagnosed before 50 years of age, 35% (7)  $<$  40 and 15% (3)  $<$  30. MyBPC3 patients had lower maximal thickness (25.15 vs. 30.45 mm, p=0.045) than those with mutations in MYH7, but higher than non-mutated patients (22.17 mm, p=0.034). Thirty percent of MyBPC3 patients had a maximal wall thickness  $\geq$  28 mm (2 of them with a thickness  $>$  40 mm were younger than 45 years old).

**Conclusions:** MyBPC3 mutations were present in 15.4% of our families. Patients with MyBPC3 gene mutations were not older at diagnosis than patients with MYH7 mutations. MyBPC3 mutations may appear in young patients with severe hypertrophy.

**P01.301****Especrum of mutations in MyBPC3 gene in 130 families with hypertrophic cardiomyopathy**

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Hypertrophic cardiomyopathy (HCM), an autosomal-dominant disorder and the leading cause of sudden cardiac death in the young, is caused by mutations in genes encoding sarcomeric proteins. One of the most common genetic causes for HCM involves mutations in MYBPC3, the gene encoding cardiac myosin binding protein C.

To determine the spectrum of mutations in MyBPC3 gene, 130 index cases were tested.

A total of 16 different mutations, including 8 novel ones, were identified in 20 families (15.4%): 9 missense (D75N,A216T,V471E,R495W,R502Q[in 2 families], E542Q[in 3 families],T957S,R1022P[in 2 families],E1179K, 4 deletions (Q327fs,K504del, K600fs,P955fs) and 3 intronic regions (IVS6+5G>A,IVS11-9G>A,IVS29+5G>A).

Ten of the mutations were identified on 7 of the 34 exons studied. Exons 16 and 17 could be "hot spots" due to the fact that 35% of the families presented a mutation in these exons. The average of age in the index cases with mutation was 50 years. Four carriers relatives weren't diagnosed HCM but they were below mean age of diagnose, so it is possible that they haven't yet developed the phenotype. Besides, there were 3 relatives without conclusive diagnostic of HCM that didn't present mutation; this could suggest mutations in other genes.

In conclusion, MyBPC3 is one of the genes most commonly affected by HCM-causing mutations which leads to a relatively mild phenotype with an adult age of onset. Furthermore, the existence of individuals with HCM but without mutation in MyBPC3, suggests the presence of additional disease-causing mutations in other genes.

**P01.302****Neuroblastoma (NB) with hypothalamic dysfunction (HD): report of a series of 14 cases**

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Neuroblastoma (NB) is a frequent paediatric tumour for which recurrent somatic rearrangements are known. Congenital central hypoven-

tilation syndrome (CCHS) is a broad dysautonomia with predisposition to Hirschsprung disease and NB in 20% and 5-10% of the cases respectively. The identification of the PHOX2B gene as disease causing in CCHS allowed to find that PHOX2B could be mutated in some familial and sporadic cases of NB also. In order to find genes involved in NB predisposition, we collected syndromic NB cases. Here we report a series of 14 patients with hypoventilation and HD. Hypothamic dysfunction is characterized by obesity, hyperprolactinemia, central hypothyroidism, disordered water balance, unresponsive growth hormone to stimulation test, corticotrophin deficiency and abnormal puberty be it precocious or delayed. Up to now, all reported cases (25) have been sporadic. The natural history of the syndrome is striking : rapid onset obesity due to hyperphagia is the first symptom, followed by hypoventilation with a mean delay of 1.5 year. Neurocognitive deceleration and mood disorders are frequently noted. The outcome remains poor in this group of patients and would benefit from early diagnosis in order to anticipate ventilation and possible metabolic disorders. Tumour predisposition is as high as 4/14 (29%) in this series. Finally, we report a familial case with recurrence in siblings and discuss a paraneoplastic syndrome an autoimmune disorder and a monogenic condition.

#### P01.303

##### **Neonatal data on 530 children born after ICSI using testicular spermatozoa**

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**INTRODUCTION:** We analysed pregnancy outcome and neonatal data of children born from a consecutive cohort of ICSI cycles performed with testicular spermatozoa.

**MATERIAL & METHODS :** Questionnaire data and results of physical examinations on 530 ICSI children from azoospermic fathers from whom testicular spermatozoa were obtained (period 1994-2007) were compared with control data from 2889 ICSI children. All children were examined by a geneticist during their first year of life.

A subanalysis of outcome parameters was performed in relation to the cause of azoospermia.

**RESULTS:** Of the 657 pregnancies obtained after ICSI using testicular spermatozoa, 426 (63.1%) were ongoing after 20 weeks leading to the birth of 530 liveborn children: 331 singletons, 178 twins and 21 triplets. Mean birthweight, length and head circumference as well as prematurity, low birthweight and perinatal mortality in singletons and twins did not differ from the ICSI control group. No increase in major malformations was observed in liveborn TESE children (4.90%) when compared to the control ICSI group. Prenatal (126) and postnatal (72) karyotype analysis revealed 2.02% *de novo* abnormalities and 0.51% inherited abnormalities compared to 0.45% *de novo* anomalies (OR 4.57; 95%CI 1.68-12.38) in the general newborn population.

Further subanalysis in relation to the cause of azoospermia did not reveal any differences in neonatal outcome parameters and major malformations rate.

**CONCLUSIONS:** Neonatal outcome and major malformations rate were similar in TESE children compared with a control cohort of ICSI children but more *de novo* chromosomal anomalies were found compared to the general newborn population.

#### P01.304

##### **A case with interstitial deletion of 11q**

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We report on a 12 year-old boy with mental retardation (EQ 45), cleft palate, umbilical and unilateral inguinal hernia and discrete dysmorphic features: low frontal hairline, red hair with multiple whorls and unusual growth pattern, flat occiput, low set ears with poorly formed helices, narrow auditory canals, full eyelids, up-slanting palpebral fissures, prominent lips, small mandible, narrow sloping shoulders, discrete pectus excavatum, inverted nipples and abnormal distribution of fat tissue over the proximal forearms. On the upper limb there were proximal placement of thumbs, restricted flexion in all metacarpo-phalangeal joints, prominent finger pads and short and broad nails and

distal phalanges. On the lower limb there were diminished stability in the knees and pes planus. The boy had a good social behaviour and pleasant nature.

Karyotype revealed an interstitial deletion involving 11q14. By arrayCGH the deletion could be further defined to 11q13.5->11q14.3.

We compared our case with case reports with similar sized deletions involving the bands 11q13 and 11q14. Similar phenotypic findings included mental retardation, normal measurements at birth, low frontal hairline, dysmorphic ears, full eyelids or ptosis, small mandible, high arched or cleft palate and minor features of the fingers, thumbs or toes. In interstitial deletion 11q the facial gestalt and the incidence of joint limitations is similar to Williams-Beuren syndrome, whereas in contrast to Williams-Beuren syndrome the retardation in speech is more pronounced and there is in general no heart defect, growth retardation or microcephaly.

#### P01.305

##### **A novel case of partial trisomy 2p in a 2-year old girl**

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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.

#### P01.306

##### **Clinicopathological study of a Infantile Systemic Hyalinosis case with a novel mutation in the CMG2 gene**

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Infantile Systemic Hyalinosis (ISH) is an autosomal recessive disorder, belonging to the wide and heterogeneous group of genetic fibromatosis, characterized by widespread deposition of hyaline material in the skin, gastrointestinal tract, muscles and glands. Mutations in the capillary morphogenesis gene-2 (CMG2), coding for a transmembrane protein with important roles in cell-cell adhesion and cell-extracellular matrix interactions, have been shown to cause ISH. We report on a patient with a severe form of ISH, confirmed by clinical and histological findings, who also displayed clear evidence of early muscle involvement, and enteropathy. Novel mutation of CMG2 gene has been found and review of the literature will be made.

**P01.307****Maternal uniparental isodisomy 20 : clinical report**

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We report on a young child, presenting with intractable feeding difficulties, and severe post-natal growth retardation required enteral tube feeding,

He was the second child, from non-consanguineous Caucasian healthy parents, born at 39 weeks of gestation and delivery was uneventful. Birth measurements were normal.

Nuchal translucency was detected during first trimester of pregnancy and prenatal investigation in amniotic fluid was performed.

Chromosome studies using standard R banding showed a tiny marker in all cells; parents refused complementary investigations.

Prenatal ultrason examination revealed moderate pyelectasy. Post-natal examination showed posterior urethral valves.

Post-natal cytogenetic analysis revealed that the marker was from chromosome 20 and contained only centromeric and pericentromeric segments.

The combination of cytogenetic findings and severe feeding difficulties made us speculate that it could be a maternal UPD 20 confirmed by molecular analysis.

Phenotype, in our case, is less severe (especially mental retardation) than those of the paternal 20q13.2-q13.3 deletions, even though major characteristics are present.

At 3 years, measurements are -5SD for length and weight, and -0.5SD for OFC. He says only a few words and walks with help.

This report demonstrates interpretation's difficulties when excentenary marker is found and allows us to be careful, and to look for UPD for markers derived from imprinted chromosomes.

**P01.308****Hypomelanosis of Ito - report of two patients**

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Hypomelanosis of Ito syndrome (HI - MIM\* 300337), is characterized by the presence of whirled hypochromic skin lesions often associated with systemic manifestations.

To date, epidemiological data on HI are limited. It appears to be the third most common neurocutaneous disease, second only to neurofibromatosis and tuberous sclerosis. Approximately three fourths of the patients with typical skin lesions have systemic manifestations. Typical skin lesions are initially demonstrated during the first year of life in as many as 70% of patients; they are noticeable at birth in 54% of patients. Prognosis depends on the patient's manifestations and complications of the disease. Patients with chromosomal anomalies are at risk for tumors.

We report two children, a 6 years female and a 5 years old male, with typical HI lesions and systemic nondermatological abnormalities (eg, mental retardation and epilepsy). Blood karyotyping was normal on both; skin biopsy revealed normal fibroblast karyotype in the girl and mosaicism for chromosome abnormality in the boy.

Despite recent advances, the genetic substrate for HI syndrome is far from homogenous and is not completely understood. Although several chromosomal abnormalities have been reported in HI the etiology remains elusive. The pattern of chromosomal aberrations and the polymorphic nature of this disease have led some to believe that HI syndrome is a descriptive term rather than a true syndrome.

Most cases are a de novo occurrence although very rare cases of familial HI following a dominant pattern have been described in the literature.

**P01.309****Misdiagnosis in patients with Joubert Syndrome**

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We report a 18 month-old boy born to a Moroccan non-consanguineous couple referred to our center for the presence of a Dandy-Walker Malformation (DWM) with hydrocephalus and intracranial hypertension, mental retardation, and cranio-facial dysmorphisms.

About a year after the birth of the proband, a second pregnancy of the couple was terminated at 20 weeks of gestation after ultrasound detection of DWM, cleft-lip and -palate and nasal root hypoplasia

The initial diagnosis of DWM in the proband and in the fetus was put into question. A control MRI performed after placement of a ventriculoperitoneal shunt at twenty months of age showed the classical "molar tooth sign", allowing us to hypothesize the diagnosis of Joubert syndrome.

Genetic testing is currently being performed.

These results indicate that in the presence of intracranial hypertension it might be difficult to diagnose the underlying brain malformation. Therefore the recurrence of DWM, especially if diagnosed only by fetal brain ultrasound, should take to rule out Joubert syndrome.

**P01.310****Novel mutation in the keratin 3 gene in an asymptomatic family with Meesmann corneal dystrophy suggests genotype-phenotype correlation**

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Meesmann corneal dystrophy (MCD, OMIM #122100) is a dominantly inherited disorder characterized by fragility of the anterior corneal epithelium and intraepithelial microcysts formation. Although the disease is generally mild and affected individuals are often asymptomatic, some suffer from recurrent erosions leading to lachrimation, photophobia and deterioration in visual acuity. MCD is caused by mutations in keratin 3 (*KRT3*) or 12 (*KRT12*) genes encoding cornea-specific cytoskeletal proteins. Seventeen mutations in *KRT12* and only two in *KRT3* have been described so far. In this study we report on a three-generation Polish family with MCD. Epithelial lesions characteristic for MCD were visualized with slit-lamp examination and confirmed by *in vivo* confocal microscopy. In the proband a direct sequencing of the PCR amplified coding regions of *KRT3* and *KRT12* revealed a novel 1493A>T heterozygous missense mutation in exon 7 of *KRT3*, which predicts the substitution of glutamic acid for valine at codon 498 (E498V). By a PCR-RFLP test the mutation was demonstrated to segregate with disease (four affected members, three non-affected) and to be absent in 100 controls from Polish population indicating that it is not a common polymorphism. Location of the E498V mutation emphasizes the functional relevance of the highly conserved boundary motifs at the C-terminus of the alpha-helical rod domain in *KRT3*.

**P01.311****A molecular diagnostic service for lacrimoauriculodentodigital (LADD) syndrome**

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Lacrimoauriculodentodigital (LADD) syndrome (Levy-Hollister syndrome) is a rare autosomal dominant condition characterised by multiple congenital abnormalities mainly affecting the lacrimal glands and ducts, salivary glands and ducts, ears, teeth and distal limb segments. In addition there may be mild facial dysmorphism, malformation of the kidney and respiratory system and abnormal genitalia. The phenotype of LADD syndrome is highly variable and can range from congenital renal disease causing death in the neonatal period, to aplasia of the

lacrimal and salivary glands (ALSG) only. This variability in phenotype has been seen within families with the same mutation.

LADD syndrome is a genetically heterogeneous disorder caused by heterozygous mutations in the *FGF10*, *FGFR2* and *FGFR3* genes. As the Oxford Molecular Genetics Laboratory already offers *FGFR2* and *FGFR3* gene testing as part of the Craniofacial service, testing for LADD syndrome was introduced in 2007.

Testing is done using bi-directional sequencing of the coding region and exon/intron boundaries of the *FGF10* gene, and bi-directional sequencing of exon 16 of the *FGFR2* gene and exon 13 of the *FGFR3* gene. This screening strategy is expected to pick up all known point mutations in *FGFR2*, *FGFR3* and *FGF10*, but not exonic deletions. An exonic deletion of *FGF10* has been reported.

Three cases have been analysed to date. The presented case has a maternally inherited c.1942G>A (p.A648T) mutation in *FGFR2* exon 16. Both mother and son have classical facial features, cup-shaped ears, variable nail dysplasia and lacrimal duct obstruction. The proband also had unilateral renal agenesis.

#### P01.312

##### Limb Body Wall Complex in a Monochorial Mono-amniotic Twin

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The limb-body wall complex (LBWC) or body stalk anomaly is a variable group of congenital defects characterized by a combination of anterior body wall defects, limb defects and/or encephalocele or encephaly. There are often additional structural defects such as urogenital anomalies and abnormalities of the cloaca.

Three pathogenic mechanisms have been proposed for this anomaly: a mechanical origin by early amnion rupture, a vascular disruption during embryonal development and an embryonic origin due to a mutation in a developmental gene.

We report of a monochorial mono-amniotic twin pregnancy with ultrasound abnormalities seen in the 13-th week. The pregnancy was terminated. Post mortem examination on the first foetus showed only one kidney, a megabladder and anal atresia. The second foetus had a severe hydrops, anal atresia, a large abdominal wall defect and absence of the external genitalia.

In our case we presume that the body stalk anomaly in both foetuses results from a mutation in a developmental gene.

This case supports the hypothesis of a possible embryonal origin of limb-body wall complex.

#### P01.313

##### Novel LMNA mutation seen in a patient with leanness, severe insulin resistance and facial dysmorphisms

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Here we describe a novel LMNA gene mutation in a 27-year-old woman with a complex disorder characterized by extreme leanness, severe insulin resistance, mixed calcific valvulopathy involving both mitral and aortic valves, slight facial dysmorphisms, low femoral bone mass. The patient is the 2nd child of non consanguineous parents. Physical examination showed: extreme leanness (BMI 15.6 kg/m<sup>2</sup>), especially at the legs, craniofacial dysmorphisms with sharp nose, small chin and small mouth, narrow palate and tongue hypoplasia, micrognathia and slight dental overcrowding; she has subtle and sparse hair. Surprisingly, whole body DEXA scan showed normal total fat mass (25.8%) and distribution, whereas MRI showed no alteration of both subcutaneous and visceral adipose tissue depots. Metabolic alterations includes hyperinsulinemia both basal and 2 hours after OGTT (respectively 57.2 and 982.4 μU/ml), insulin resistance assessed by glucose clamp (M=2.3 mg/kg/min). LMNA sequencing evidenced a novel heterozygous missense mutation in exon 4 that replaces well-conserved residue glutamic acid at position 262 to lysine (p.E262K, c.784 G→A). Her parents were negative for the same mutation, suggesting that this mutation has de novo origin. Sequence analysis of 100 healthy normal controls did

not reveal this sequence alteration, indicating that this mutation is not a common variation. Genetic screening of additional genes mutated in laminopathic or lipodystrophy disorders (*ZMPSTE24*, *PPARγ BSCL2*) showed a wild type sequence. This study extend the large phenotypic spectrum of laminopathies and may lead us better understanding molecular basis of this group of disease.

#### P01.314

##### Serendipitous detection of ENG haploinsufficiency in a girl with nail-patella syndrome and a 9q33.3-q34.11 microdeletion

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We describe the first case of a microdeletion syndrome with concurrent loss of the LMX1B and ENG genes on 9q33.3-q34.11. The proposita, first-born of healthy non-consanguineous parents, was born at term of a pregnancy complicated by hypertensive gestosis and IUGR. She had a severe psychomotor delay and, during infancy, myoclonic epilepsy and generalized seizures. Nail-patella syndrome was diagnosed upon physical and radiological examination. She had occasional nose-bleeding episodes since the age of 6 years. No ocular neither renal function alteration have been detected until the current age of 14 years. Precocious puberty was blocked with triptorelin treatment until the age of 12 yrs: regular menses began six months after therapy withdrawal.

aCGH was performed to look for a common cause of the phenotypic manifestations. A de novo 1.7 Mb deletion in 9q33.3-q34.11 was detected. Microsatellite analysis revealed that the deletion was of maternal origin. The deleted region includes the LMX1B and ENG genes. Deletion of LMX1B is responsible for manifestations of the nail-patella syndrome displayed by the patient. ENG haploinsufficiency causes hereditary hemorrhagic telangiectasia (HHT). At present, the only clinical manifestation of HHT present in the patient is nose bleeding. This observation underscores the predictive power of aCGH. This technique does not only allow to clarify the origin of existing clinical manifestations but may also lead to the identification of haploinsufficiency for genes causing adult-onset conditions with implementation of appropriate surveillance.

#### P01.315

##### Discordant monozygotic twins suggests that macrocephaly-capillary malformation is a post-zygotic event

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Macrocephaly-Capillary Malformation (M-CM) is the new name for Macrocephaly-cutis marmorata telangiectatica congenita.

We describe a 17 month old girl with M-CM. She has a healthy monozygotic twin. She is the fourth of five girls from second degree consanguineous Turkish parents.

The proband was born at 35 weeks of gestation by caesarean-section for foetal distress. Birth weight was 2,47 kg (P50), birth length 46 cm (P25) and occipito-frontal circumference (OFC) 35,5 cm (>P90). During the neonatal period profound hypoglycaemias were noted. Clinical examination shows: frontal bossing, nasal and frontal angiomas, strabismus of right eye (inconstant), hypertelorism, right epicanthus, ogival palate, abnormal palmar crease, gap between first and second toes, axial and peripheral hypotonia, cutis marmorata, thick subcutaneous tissue. She has psychomotor retardation and macrocephaly. The cerebral MRI at 7 months showed cerebellar tonsillar herniation with hydrocephaly and signs of cerebral hypertension. She had ventricular shunting at 11 months of age with improvement of macrocephaly and psychomotor retardation. Thermoregulation trouble appeared after one year of age with usual temperature between 38-39°C.

The karyotype and X-inactivation pattern are normal. Twin zygosity testing performed on buccal smear DNA proved monozygosity.

There are about 100 published cases of M-CM. Detailed clinical findings and cerebral imaging of our case are similar to those reported before. Thermoregulation trouble has never been described in M-CM. All the cases of M-CM are sporadic. Our patient is a unique case of M-

CM in monozygotic twins where only one is affected, suggesting that M-CM is due to a post-zygotic event.

#### P01.316

#### **Study of the aortic risk associated with *FBN1* mutations from an international study of 1013 patients with Marfan syndrome or related type I fibrillinopathy**

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In order to better describe the aortic risk associated with mutations in the *FBN1* gene, we took advantage of a series of 1013 genotyped probants carrying Marfan syndrome or another type I fibrillinopathy, recruited for a genotype-phenotype correlation study. At 40 years of age, the cumulative probability of presenting an ascending aortic dilatation (AAD) was 75% [95%-CI=72%-78%], 26% [95%-CI=22%-31%] for ascending aortic dissection and 41% [95%-CI=36%-46%] for cardiac surgery. The cumulative probability of presenting AAD was higher in males than in females (80% [95%-CI=76%-83%] at 40 years of age in males versus 70% [95%-CI=65%-75%] in females; p=0.0036). An higher risk of aortic surgery was also observed in males (46% [95%-CI=39%-52%] versus 34% [95%-CI=27%-42%] in females; p=0.0002). The study of the paediatric cohort (n=320) showed that aortic complications remained an exception, with a 1% risk of aortic dissection and a 5% risk of aortic surgery at 10 years of age. Adult patients diagnosed during childhood had a higher risk of developing AAD (81% [95%-CI=73%-88%] at 40 years of age) than patients diagnosed in adulthood (65% [95%-CI=60%-69%], p<0.0001). They also had a higher risk of aortic surgery (64% [95%-CI=52%-77%] at 40 years of age compared to 35% [95%-CI=30%-40%], p<0.0001). The search for clinical factors associated with the occurrence of ascending aortic dilatation over time revealed that patients with mitral valve prolapse (HR=5.86; IC-95%=3.08-11.18) or ectopia lentis (HR=2.17; IC-95%=1.55-3.05) had a higher risk of developing AAD.

#### P01.317

#### **Case report: a boy with mild mental retardation, hypogenitalism and karyotype 46,XY,add(8)(q24.3) de novo**

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We report on 27-month-old boy with abnormal karyotype. He was born premature at 35-36 weeks of the fifth gestation as the first child of healthy non-consanguineous parents. At birth, the mother was 31 years old and the father was 59 years old. They both had a professional contact with industrial hazards. Being married first time, the mother had three miscarriages. In her second marriage, she had a pregnancy ended as spontaneous abortion at six weeks of gestation. The fifth pregnancy was complicated with anemia, pyelonephritis, threatened miscarriage at 12 weeks of gestation. Results of prenatal ultrasound and biochemical screening were normal. The proband presented with a birthweight of 2350g, length of 44 cm, at 12 months his weight was 10200g, length 81cm. Findings at 27 months included mild mental retardation, speech and developmental delay, asthenic stature, telecanthus, strabismus alternating, flat nasal bridge, geographical tongue, unilateral simian crease, broad hallux, flat-valgus feet, moderate hypotonia and hyperflexibility of the distal parts of the hands and feet, diastasis of rectum abdominal muscles, small testes (<1 ml), hypoplastic penis. The chorionic gonadotrophin test showed testosterone increased (initial level-2.2nmol/l, after ChG-27, normal range 8-38). TSH

level was 3.56 microME/ml (normal range 0.1-3.5), FT<sub>4</sub>-9.2 picomol/l (normal range 9-28). Cytogenetic testing revealed the proband to be a carrier of 46,XY,add(8)(q24.3) while his parents had normal karyotype in the blood. For uncovering the origin of the additional region on chromosome 8, a multi-tissue screening for the presence of mosaicism for the abnormal cell line is advisable.

#### P01.318

#### **Microcephalia vera : cognitive profile and neuroradiological presentation of 9 children with *ASPM* gene mutations**

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Primary microcephaly (MIM 251200) is a neurodevelopmental condition in which there is a global reduction in cerebral cortex volume. Abnormal neuronal and glial proliferation lead to microcephalia vera (MV) and microcephaly with simplified gyral pattern (MSG). Patients affected by MV or MSG show mental retardation. The MCPH5 locus for MV patients on 1q31 corresponds to the *ASPM* gene, a human ortholog of the *Drosophila melanogaster* 'abnormal spindle' gene (*asp*), identified by homozygosity mapping and found responsible of roughly ½ of the MV cases in all ethnic backgrounds.

We report on clinical-genetical results from a multicentric european study on MV. Nine out of 70 patients with a diagnosis of MV or MSG (based on clinical and neuroradiological studies) showed mutations in the coding sequence of *ASPM*. Patients' IQ ranged from 35 to 70. One third of them had MSG, one an unilateral polymicrogyria. We observed that MR was more severe in patients who presented a more severe microcephaly and a MSG rather than MV. Furthermore, memory was better preserved than attention or fine motor functions. MV and MSG represent the phenotypical spectrum of *ASPM* gene mutations.

#### P01.319

#### **Multiple minor congenital defects associated with autism spectrum disorders**

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Autism spectrum disorders represent a wide spectrum of conditions characterized by abnormalities of social interactions and communication, severely restricted interests and highly repetitive behavior. They have multiple genetic and nongenetic causes. In order to ascertain the cause and the onset of the disorder, establishing a positive correlation with some markers would be useful. Minor congenital defects result from either genetic or environmental causes that act during pregnancy and are an indirect measure of abnormal embryo and fetal development. The purpose of the study was to see if patients with autism spectrum disorders have more minor congenital defects than healthy individuals and if so, if this finding correlates with the history of the patients. The incidence and media of minor congenital defects were determined in 76 patients with autism spectrum disorders and in unrelated control subjects matched by age and sex. The frequency of minor anomalies was not significantly different in the two groups, 14.47% in autistic children and teenagers, compared to 14.80% in healthy controls. The mean number of minor congenital anomalies was significantly higher in the autistic group, 3±0.70 minor defects/patient, compared to 1.43±0.36 minor defects/patient in controls. None of the control subjects had three or more minor anomalies. These results support the idea that minor defects, especially multiple ones, may represent markers of early prenatal factors that contribute to the adverse outcome.

**P01.320****Gene Copy Number analysis using semi-quantitative multiplex PCR-based assay on capillary electrophoresis systems****S. Jankowski, E. Currie-Fraser, L. Xu;***Applied Biosystems, Foster City, CA, United States.*

Deletions and duplications in genomic DNA have been implicated as pathogenic mutations in many diseases. Traditionally, detection of these types of mutations is done using southern blot hybridization or Fluorescence in situ hybridization, techniques which can be laborious, time-consuming and require high quantities of starting material. In this study we present analysis of a semi-quantitative multiplex PCR-based method that uses relative quantitation of fluorescently-labeled fragments. Fragments from BRCA1, BRCA2, 9p21 and MMR (MSH2) regions were tested using labeled probes from DNA that had been isolated from blood. Amplified samples were then run on an Applied Biosystems capillary electrophoresis platform and the data was analyzed in GeneMapper® software v4.0. After signal normalization, loci regions that had undergone deletions or duplications were identified using the GeneMapper® software v4.0 report manager feature and verified using the dye scale functionality. Our results will highlight an easy to use, optimal workflow and analysis that can be used for both small and large-scale studies.

**P01.321****A severe form of the X- linked microphthalmia with linear skin defects syndrome (MLS) in a female newborn - expansion of the clinical spectrum****E. Steichen-Gersdorff<sup>1</sup>, F. Pientka<sup>2</sup>, D. Kotzot<sup>3</sup>, I. Wimberger<sup>2</sup>, E. Griesmaier<sup>1</sup>, K. Kutschke<sup>2</sup>;**

<sup>1</sup>Medical University of Innsbruck, Innsbruck, Austria, <sup>2</sup>Institute for Human Genetics, Hamburg, Germany, <sup>3</sup>Department of Medical Genetics, Innsbruck, Austria. The microphthalmia with linear skin defects syndrome (MLS/MIDAS) is an X-linked disorder with male lethality, mostly associated with segmental monosomy of the Xp22 region. MLS is characterized by unilateral or bilateral microphthalmia and linear skin defects limited to the face and neck. We report a female newborn with a severe phenotype of MLS syndrome. She presents with the typical MLS signs, such as bilateral microphthalmia, primary persistent vitreous and sclerocornea, and a linear erythematous skin defect on her cheek. Additional features include agenesis of the corpus callosum, left-sided diaphragmatic hernia, an ileal duplication cyst, imperforate anus with a rectoperitoneal fistula, a hamartoma at the right liver lobe, and partial duplication of the uterus.

A terminal deletion of the short arm of one of her X chromosomes was confirmed by fluorescence *in situ* hybridization using various BAC clones mapping in Xp22.3-p22.2. The breakpoint was localized in Xp22.2 and the size of the deletion was estimated to cover ~11 Mb, including the HCCS gene. Mutations in HCCS, encoding the mitochondrial holocytochrome c-type synthase, have recently been identified in patients with MLS and normal karyotype. Midline defects, as imperforate anus and duplication of the uterus, have rarely been described in patients with MLS. However, anal atresia is a frequent feature in male patients with X-linked Opitz G/BBB syndrome carrying a mutation in MID1. We hypothesize that deletion of both MID1 and HCCS as well as skewed XCI might have contributed to the complex disease phenotype of our patient.

**P01.322****Corpus Callosum Agenesis In Three Patients With Moebius Syndrome****S. Basaran Yilmaz, E. Karaca, G. Yesil, A. Yuksel;***Istanbul University,Cerrahpasa Medical Faculty, Department Of Medical Genetics, Istanbul, Turkey.*

Moebius Syndrome is a rare disease which is defined as congenital facial palsy with impairment of ocular abduction. The facial nerve(7) and abducens nerve(6) are most frequently involved, but other cranial nerves may be involved as well. Other variable features include orofacial dysmorphism, limb malformations, mental retardation, external ear defects. The most accepted hypothesis with regard to pathogenesis is that disruption of the primitive subclavian arteries and their branches before establishment of a sufficient blood supply to the brain stem leads to the symptoms of Moebius sequence. Up to date congenital anomalies of the posterior fossa, including Arnold-Chiari malformation,

pineal cyst, hypoplastic cerebellum, asymmetric lateral ventricles, anomalies of cranial nerves nuclei had been described.

We describe here 3 cases with Moebius Syndrome in two of whom corpus callosum agenesis were detected.

The patients referred to our clinic with facial asymmetry and additional dysmorphic features. Cranial MRI revealed corpus callosum agenesis in two of the patients.

To our knowledge, these are the first patients with Moebius Syndrome who are reported presenting corpus callosum agenesis.

**P01.323****MOMO syndrome without macrosomia - alternative definition of the obesity syndrome****N. Tyshchenko<sup>1</sup>, T. Neuhann<sup>1</sup>, E. Schrock<sup>1</sup>, A. Huebner<sup>2</sup>, S. Tinschert<sup>1</sup>;**

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Two unrelated patients with a combination of macrosomia, macrocephaly, obesity and ocular abnormalities (retinal coloboma and nystagmus) were described by Moretti-Ferreira et al. (1993). Thereafter, the new syndrome - called MOMO syndrome (Macrosomia, Obesity, Macrocephaly, and Ocular abnormalities) - was categorized as an overgrowth syndrome. However, since a third patient, published in 2000, was of short stature, overgrowth was discussed as non-mandatory for the diagnosis.

We report a further patient with proposed diagnosis MOMO syndrome. At the age of 5 years and 7 months the girl presented with overweight, macrocephaly and borderline short stature. She showed a prominent forehead, deep-set eyes, a flat nasal bridge, anteverted nares, a cupid bow upper lip, low-set posteriorly rotated ears and tapering fingers. She had hypotonia, recurrent febrile convulsions and developmental delay. A hand radiogram showed delayed bone age. Ophthalmological examination revealed a choroid coloboma in her left eye. Metabolic tests and chromosomal examinations including Array-CGH analysis with the 44k Operon chip were normal. On the basis of the general obesity, macrocephaly, coloboma of the choroid and mental retardation the diagnosis of MOMO syndrome was made. The comparison of these four patients showed that a combination of obesity, macrocephaly, ocular coloboma (especially coloboma of the retina or choroid) and mental retardation is specific for MOMO syndrome. Due to variability of stature anomalies we propose to form the acronym "MOMO" from Macrocephaly, Obesity, Mental retardation and Ocular abnormalities, excluding macrosomia from the syndrome name.

**P01.324****Mosaic trisomy 22: report of two patients and review of the literature****M. Rio, S. Noel-Couillard, M. Le Merrer, M. de Blois, O. Raoul, V. Malan, A. Munnich, M. Vekemans;***Department of genetic, paris, France.*

Non-mosaic trisomy 22 is commonly seen in spontaneous abortions and is incompatible with life. Conversely, mosaic trisomy 22 is observed in newborns and is compatible with long survival. The clinical presentation of mosaic trisomy 22 is variable. It includes growth retardation, mental retardation, limb malformation, congenital heart defect and dysmorphic features. Here we report two unrelated patients with mosaic trisomy 22.

Both patients were referred for genetic evaluation due to developmental delay and failure to thrive. Patient 1 had intrauterine and post natal growth retardation, microcephaly, complex heart defect, severe developmental delay, facial dysmorphism, deafness, skin pigmentation anomalies, brachydactyly, short toes with syndactyly. Interestingly, patient 2 had an overlapping phenotype including post natal growth retardation, non compaction of left ventricle, developmental delay, deafness, and skin pigmentation anomalies. Facial dysmorphism, hand and feet anomalies were also similar to the one observed in patient 1. Routine chromosome analysis on lymphocytes showed normal karyotype in both patients. Chromosome analysis of fibroblasts showed a mosaic trisomy 22: 47,XY,+22[12]/46,XY [15] in patient 1, 47,XX,+22[17]/46,XX[2]. These findings were confirmed by *in situ* hybridization. The phenotypic spectrum observed in both patients is compared to previously reported cases.

**P01.325****Mowat-Wilson syndrome in a patient with severe eye anomalies**A. Medeira<sup>1</sup>, G. Black<sup>2</sup>, E. Seabright<sup>2</sup>, I. Cordeiro<sup>1</sup>;<sup>1</sup>Serviço de Genética Médica, Hospital de Santa Maria, Lisboa, Portugal,<sup>2</sup>Department of Clinical Genetics, St. Mary's Hospital, Manchester, United Kingdom.

Mowat-Wilson Syndrome (MWS) is a mental retardation syndrome associated with distinctive facial features, frequent microcephaly and epilepsy, and a variable spectrum of congenital anomalies, including Hirschprung disease (HSCR), corpus callosum agenesis, genitourinary and heart defects. Eye anomalies such as strabismus, microphthalmia, colobomas, have been reported in a few patients.

Heterozygous mutations or deletions involving the zinc finger homeobox 1B gene (ZFXH1B) cause MWS

We report a 5 years old female patient with severe psychomotor delay, epilepsy, microcephaly, deep set eyes, left eye anophthalmia, right eye severe microphthalmia with retinal and optic nerve colobomas, broad nose with long columella, short nasolabial distance, large mouth, prominent chin, large ears with fleshy uplifted lobes, septo-optic dysplasia, corpus callosum hypoplasia, HSCR, ventricular septal defect. Mutation analysis of ZFXH1B gene identified a truncating mutation c.2083C>T[p.Arg695X].

MWS was originally reported as a syndromic form of HSCR. It is now known that it frequently occurs without HSCR and can be recognized by a consistent facial phenotype associated with severe mental retardation. MWS and Goldberg-Shprintzen may overlap features but faces are distinct. Some MWS patients have an ataxic gait and smiling personality suggesting Angelman, but the characteristic facial features of MWS and the presence of other congenital anomalies usually distinguish these conditions. The patient that we report presents the main manifestations described in MWS including facial phenotype. Eye anomalies have been reported in MWS, but not as severe as in our patient. As far as we are aware this is the only MWS patient with anophthalmia.

**P01.326****Recurrence of Mowat-Wilson syndrome in two siblings carrying a novel mutation in the ZEB2 gene**M. Cecconi<sup>1</sup>, F. Forzano<sup>1</sup>, L. Garavelli<sup>2</sup>, M. Grasso<sup>1</sup>, E. Di Maria<sup>1,3</sup>, F. Faravelli<sup>1</sup>;<sup>1</sup>Galliera Hospital, Genova, Italy, <sup>2</sup>Clinical Genetics Unit, S. Maria Nuova Hospital, Reggio Emilia, Italy, <sup>3</sup>Dept. of Neuroscience, Ophthalmology and Genetics, University of Genova, Genova, Italy.

The eponym Mowat-Wilson syndrome (MWS, OMIM #235730) designates a multisystem congenital disease caused by heterozygous mutations in the ZEB2 gene. Molecular diagnosis has helped to delineate the cardinal signs (facial gestalt and delayed psychomotor development) as well as several variously associated congenital disorders. To date, literature describes approximately 180 patients with MWS. Only three cases of recurrence in siblings were reported.

We describe two sisters with clinical features of MWS. Antenatal scanning in the first sibling had revealed corpus callosum agenesis. Hypotonia and feeding difficulties were present in the neonatal period. The psychomotor development was delayed. US scans of the heart and abdomen were normal. At our evaluation (5 years of age), facial dysmorphisms strongly suggestive of MWS were noted. The second born had a congenital complex heart disease. The surgical interventions on heart defects were complicated by renal failure, airways infection, denutrition and a cardiac arrest. At our evaluation (3 yrs.), she presented microcephaly and facial dysmorphisms, and with the sequelae of cerebral anoxia.

Direct sequencing of ZEB2 revealed in both patients a novel heterozygous c.310C>T transition, resulting in a stop codon (p.Q104X). The nucleotide substitution was not found in both parents, as well as in 94 unrelated control individuals. No evidence of somatic mosaicism was found in the parents.

In light of this and previous reports, the recurrence risk provided to families with an isolated MWS case should be frequently revisited. Genetic counselling should consider intrinsic clinical variability, risk of complications and still inaccurate empiric risk of recurrence.

**P01.327****The placenta as the main clue to the diagnosis in a patient with recurrent nonimmune hydrops fetalis**A. Moresco<sup>1</sup>, N. Mazzitelli<sup>2</sup>, N. Ronaldo<sup>3</sup>, D. Caceres<sup>3</sup>;<sup>1</sup>Centro Nacional de Genética Médica, Buenos Aires, Argentina, <sup>2</sup>Unidad de Patología. Hospital Materno Infantil Ramón Sardá, Buenos Aires, Argentina,<sup>3</sup>Servicio de Neonatología. Hospital Materno Infantil Ramón Sardá, Buenos Aires, Argentina.

Introduction: Nonimmune hydrops fetalis (NIHF) usually carries a poor prognosis and can have diverse causes. Lysosomal storage diseases (LSD) are among the causes of NIHF.

Objective: Report a case of recurrent NIHF where the placental histology lead to the investigation of Mucopolysaccharidosis VII (MPS VII).

Materials and methods: Histopathological examination of the placenta and serum beta-glucuronidase (GUSB) activity were performed.

Case report: In the second pregnancy of non-consanguineous parents fetal hydrops was detected by an ultrasound scan at 22 weeks of gestation. An earlier pregnancy resulted also in an hydropic fetus. There was no evidence of immune hydrops, congenital infection or cardiovascular abnormalities and a normal karyotype (46,XY) was obtained. The pregnancy resulted in a depressed, hydropic infant, which despite of intensive care died at 3 days of age.

The histopathological examination of the placenta, presented vacuolated Hofbauer cells, which were positive stained with Alcian Blue and negative to PAS reaction. A normal appearance of the cytotrophoblast was noted. The electron microscopic reflected the empty appearance of the vacuoles. This features suggested a LSD with accumulation of an acid mucopolysaccharide probably MPS-VII. The diagnosis was confirmed GUSB activity that was negligible.

Conclusion: The placental histopathological examination performed in this case of recurrent NIHF led to the suspicion of MPS-VII. The diagnosis necessary had to be confirmed with the quantification of enzyme activity. The placental proved to be an useful tool, in order to limit the diagnostic resources. The accurate diagnosis is important for adequate genetic counselling.

**P01.328****Novel mutation in epithelial sodium channel alfa subunit (SCNN1A) in a patient with a cystic fibrosis-like syndrome.**M. Bernal<sup>1</sup>, F. Mora<sup>1</sup>, J. F. Rodríguez-Gutiérrez<sup>1</sup>, N. Hernández-Trujillo<sup>2</sup>, J. A. Brieva<sup>1</sup>, A. Sampalo<sup>1</sup>, A. Nieto<sup>1</sup>;<sup>1</sup>Laboratorio de Diagnóstico Molecular. Hospital Puerta del Mar, Cádiz, Spain,<sup>2</sup>Servicio de Pediatría. Hospital Puerta del Mar, Cádiz, Spain.

A six months old girl clinically diagnosed of cystic fibrosis was admitted for molecular study of CFTR gene. No mutations were detected using Inno-Lipa CFTR19 and CFTR17+Tn kits. A subsequent complete screening using DGGE and SSCP/HD was also negative (detection level of 97%). In the search for an alternative diagnosis, an extremely high aldosterone and renin activity levels were found suggesting the diagnosis of a multisystem primary type I pseudohypoaldosteronism. This is a rare autosomal recessive disease that results from mutations in the genes encoding epithelial sodium channel subunits (alfa, beta, gamma). Most mutations have been described in the alfa subunit gene (SCNN1A). This gene was sequenced in the patient and her parents. A C>A substitution at position 301 resulting in a change of glutamine for lysine at amino acid 101 (Q101K) was found. The patient was homozygous for the mutation while parents were both heterozygous. This mutation has not been reported to date. Residue 101 is at the first membrane-spanning segment of the channel inside a structural motif that has been shown to be essential to proper channel folding or assembly. In addition the 101Q residue is highly evolutionarily conserved. This data strongly suggest that Q101K mutation affects channel function and support the diagnosis of multisystem primary pseudohypoaldosteronism in this patient.

**P01.329****Mulvihill-Smith Syndrome - Case Report**

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Mulvihill-Smith syndrome is a very rare autosomal dominant disorder, defined by low birth weight, dwarfism, a prematurely aged facial appearance, pigmented naevi, hearing impairment and mental retardation.

We report a case in order to illustrate the clinical features of this progeroid disease. The proband is a 13 years old male that presents: short stature (-2.3 SD) and microcephaly (-3.7 SD; without bulging sutures). Clinical examination also reveals: poorly muscled build with lipoatrophy, thin skin with pigmented naevi, small prematurely aged face with pointed chin and retrognathia, small mouth and total anodontia (confirmed by radiologic examination). The patient has mild mental retardation, speech defect and hoarse voice. Family history is positive for anodontia (the mother has no teeth).

We made differential diagnosis with Leopard syndrome and other progeroid syndromes.

In conclusion, we present a male with Mulvihill-Smith syndrome in order to illustrate a rare disorder and to discuss the importance of clinical features like dwarfism, anodontia and pigmented naevi for diagnosis.

#### P01.330

##### Nail patella-syndrome: Phenotype and genotype correlation

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Nail patella-syndrome (OMIM: 161200) is a rare autosomal dominant connective tissue disorder with a prevalence 1 in 50 000. The phenotype is associated with multiple deformities affecting the nails, skeletal system, kidneys, and eyes. Skeletal features include absent or hypoplastic patellae, patella dislocations, scoliosis, elbow abnormalities, talipes and iliac horns. Inter- and intrafamilial clinical variability are common in this disorder particularly for skeletal abnormalities, presence and severity of nephropathy and ocular anomalies like glaucoma. Mutations in the gene encoding the Lim Homeo Box Transcription Factor-1 (LMXB1), mapped on chromosome 9 (9q34), are responsible for the clinical phenotype of NPS. No clear genotype-phenotype association have been found to date by the published cases.

We present here the phenotype and genotype of two different NPS Families. First family presented an isolated case and the second one a dominantly inherited three generations NPS family with multiple affected family members. The main clinical symptoms were lower limbs disabilities and nail dysplasia with remarkable inter- and intrafamilial variability. Neither nephropathy nor ocular problems were found in our all examined cases. Molecular analysis of the LMXB1 showed a de-novo wide deletion in intron 2 by the first family and an inherited heterozygous deletion in exon 3 (430delG) in the second Family.

Genetic counseling and follow-up regarding the different manifestations are important for all family members who have inherited the disease but could have a milder phenotype and remain underdiagnosed.

#### P01.331

##### Clinical characterisation of NBIA patients with and without mutation in PANK2 gene (PKAN)

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Neurodegeneration with Brain Iron Accumulation (NBIA) is a heterogeneous group of disorders sharing the underlying and by MR imaging detectable feature of iron accumulation in the basal ganglia. A substantial part of these patients have mutations in PANK2 (Pantothenate Kinase-Associated Neurodegeneration, PKAN). NBIA patients with and without PANK2 gene mutations share common clinical symptoms like dystonia, parkinsonism, pyramidal signs, cognitive deficits and dysarthria. Analysis of clinical symptoms of NBIA patients is often difficult because of the small patient numbers and several different investigators involved. We took advantage of a large collection of 43 NBIA patients characterized by a single investigator (T. K.). Screening PLA2G6 not mutations were found, in 24 patients two mutations have been identified in PANK2. The eye of the tiger sign were found in the majority of patients (21 out of 24) with PANK2 mutation. The group of patients with PANK2 mutations can be distinguished from the group without mutation by age of onset, loss of gait, the occurrence of generalized dystonia, parkinsonism, cognitive deficits, oromandibular dystonia and dysarthria.

#### P01.332

##### Cytogenetic and molecular analysis of patients with Nijmegen breakage syndrome in Serbia

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Nijmegen breakage syndrome (NBS) is a rare autosomal recessive chromosome instability disorder, characterized by microcephaly, growth retardation and elevated risk of cancer. Cells cultured from NBS patients exhibit an increased cellular hypersensitivity to specific mutagenic agents such as bleomycin (BLE). It was shown that patients with NBS have mutations in the *NBN* gene, which is involved in the sensing and repair of DNA double strand breaks. Most of the known NBS patients so far are of Slavic origin and share the founder *NBN* mutation, 657del5.

Here we report on five Serbian children with NBS, diagnosed and treated at the Mother and Child Health Care Institute of Serbia from July 2005 to September 2007. Cytogenetic analyses were performed on control and BLE (1µg/ml) - treated, peripheral blood cultures using standard procedure. Metaphases were examined for chromosome breaks and aberrations. Three cultures were successfully established revealing increased chromosome breaks (0.27-0.81 vs. 0.02-0.05 breaks/cell) and various aberrations including chromosomes 7 and 14, with significant difference as compared to their control counterparts. Molecular analysis for the presence of the 657del5 mutation was carried out in five patients on DNA samples extracted from peripheral blood, using modified PCR method and heteroduplex analysis on PAGE gel. Homozygosity for the 657del5 mutation was detected in all analyzed NBS children.

Patients described here are the first Serbian NBS patients reported so far. Considering that NBS patients are extremely sensitive to ionizing radiation an early and precise cytogenetic and molecular diagnosis is very important for the management of the disease in case of malignancy.

#### P01.333

##### Recurrent severe pulmonary manifestations in 6 patients with nodular periventricular heterotopia associated and FLNA mutation

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Nodular periventricular heterotopia is an X linked dominant neuronal migration disorder caused by mutations in the filamin A (FLNA) gene. FLNA is an actin-binding protein and has a role in the modulation of cell shape and migration. A high phenotypic diversity has been described in association with FLNA mutations, from nodular periventricular heterotopia to otopalatodigital syndrome and frontometaphysal dysplasia. Extra neurological features have sometimes been described in patients with nodular periventricular heterotopia, including patent ductus arteriosus, coagulopathy, or Ehlers Danlos phenotype. In this study, we reported on 6 females aged 2 to 48 years, presenting with nodular periventricular heterotopia, FLNA gene mutation and notable lung manifestations. The median age of onset of lung manifestations was 4.5 months (1.5-240). Manifestations included recurrent early-onset pneumopathies in 5/6 patients, recurrent bronchiolitis in 4/6 patients and asthma in 4/6 patients. 5/6 patients had to be hospitalized at least once for lung involvement, including one-year cumulative length of hospitalisation in one child and hospitalisation in intensive care in another. Long-term oxygen dependence was described in 2 patients. The symptomatology appears to improve with age. We believe that such lung phenotype can be a recurrent manifestation in patients with nodular periventricular heterotopia, consequence of the mutation in the FLNA gene. This hypothesis is supported by the recent demonstration of interaction between FLNA and CFTR, the ubiquitous distribution of FLNA and its role in the cytoskeleton.

**P01.334****Identification of a novel NOTCH1 mutation in patient with bicuspid aortic valve disease and thoracic aortic aneurysm**

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The bicuspid aortic valve (BAV) (MIM#109730) is the most common congenital cardiac malformation, occurring in 1-2% of the population. This condition is associated with a significantly increased risk of developing thoracic aortic aneurysms and acute aortic dissection. The valve calcification often observed in BAV is a result of inappropriate activation of osteoblast-specific gene expression. Recently, an association between mutations in NOTCH1 gene and aortic valve disease has been described. NOTCH1 encodes for a transmembrane protein that activates a signaling pathway with an active role in cardiac embryogenesis, including aortic and pulmonary valve development.

A 52-year old male was referred to our attention for suspected Marfan Syndrome. The patient did not fulfil Ghent criteria for Marfan Syndrome. He presented dilatation of the aortic root, the ascending aorta, and bicuspid and calcified aortic valve.

The family history was positive for autosomal dominant aneurysm of the ascending aorta not associated with other syndromic features.

The candidate gene NOTCH1 was screened by DHPLC and sequencing of heteroduplex amplicons.

A novel c.C4104T transition predicting the missense p.Ala1343Val mutation was identified in exon 25.

This result brings the attention of clinical geneticists to evaluate the NOTCH1 gene in patients with early onset of calcific and bicuspid aortic valve as plausible causative gene in the light of preventive surgery for carriers of gene defects and mild to severe aortic root dilation. Furthermore, this gene should be carefully evaluated in patients with thoracic aneurism dissection without syndromic features previously genotyped for TGFBR1 and TGFBR2 genes with negative results.

**P01.335****R229Q and A284 mutations of NPHS2 gene likely cause of steroid resistant nephrotic syndrome**

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The idiopathic nephrotic syndrome is a clinical pathologic entity occurring mainly in children and is characterized by massive proteinuria, hypoalbuminemia, hyperlipidemia, edema, and minimal glomerular changes. In some instances, renal biopsy may show focal segmental glomerulosclerosis (FSGS) or diffuse mesangial proliferation. In contrast to 2 types of hereditary nephrotic syndrome previously identified, congenital nephrosis of the Finnish type (OMIM 256300) and diffuse mesangial sclerosis (OMIM 256370), idiopathic nephrotic syndrome was generally regarded as a sporadic disease although a few familial cases had been reported. Most patients with idiopathic nephrotic syndrome respond to steroid therapy and show a favorable outcome. However, 20% are steroid-resistant, with progression to end-stage renal failure in many cases. The nephrotic syndrome may recur after renal transplantation in such cases. NPHS2 mutations will be found in sporadic cases of steroid-resistant idiopathic nephrotic syndrome, which represents an important cause of childhood end-stage renal disease. The detection of NPHS2 mutations is of clinical utility as it would prescribe against unnecessary immunosuppressive therapy.

A 12-year-old girl was referred to our laboratory with a diagnosis of steroid-resistant nephrotic syndrome from pediatric nephrology department. We have analyzed PCR amplicons of each of the eight exons of NPHS2 by direct sequencing and we have found that she was carrier of two mutations: R229Q and A284V. The R229Q mutation alone is insufficient to cause FSGS but the presence of a second A284V NPHS2 mutation on the other allele suggests that compound heterozygosity for these sequence changes is the likely cause of disease.

**P01.336****Mutational spectrum of BCOR gene in Oculo-Facio-Cardio-Dental (OFCD) syndrome**

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**Background:** OFCD syndrome is characterized by ocular defects (congenital cataract, microphthalmia), facial dysmorphia, congenital cardiac defects, dental irregularities (radiculomegaly, oligodontia) and skeletal deformities. It results from mutations in BCOR gene localized on Xp11.4. All affected individuals are females and the few reported mutations are either deletions or generate premature stop codons. A single missense variant was identified within BCOR in a family with Lenz microphthalmia syndrome. OFCD syndrome has been shown to encompass defects of laterality, including the heart and other viscera.

**Methods:** We analyzed BCOR gene in 26 female patients diagnosed with OFCD syndrome, 21 males with Lenz microphthalmia, 96 patients with isolated microphthalmia and 96 patients with cardiac laterality defects.

**Results:** Mutations were identified for the 26 patients with OFCD syndrome, and one patient with "isolated" microphthalmia. The same previously reported missense variant was identified in a boy with Lenz microphthalmia syndrome. No mutation was identified in the other patients tested. We report somatic mosaicism of BCOR anomalies in two families with OFCD syndrome. In both families, the mothers had 50% mosaicism in leucocytes, and their daughters carried the mother's mutation homogeneously. In the first family the mother, her monozygotic twin sister and her daughter were affected. In the second family, the daughter was affected but not her mother.

**Conclusion:** We report 27 novel patients with OFCD syndrome and mutations in BCOR gene. Cataract and radiculomegaly seem to be the two constant clinical signs in OFCD syndrome. Mosaicism in two of our families advocates cautiousness in genetic counselling.

**P01.337****Clinical heterogeneity in cases of oral clefts with multiple congenital anomalies**

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The causes of oral clefts (OCs) are multiple and complex, but the first step in the search of genetic basis should be epidemiological and clinical data. Although cleft palate (CP) is usually regarded as distinct defect from cleft lip with or without cleft palate, there is still an open question if the latter should be considered as variants of the same defect or should be divided into groups of cleft lip only (CL) and cleft lip with cleft palate (CLP). In the retrospective study of multiple congenital anomalies (MCA) of unknown origin in cases of oral clefts in Lithuania the incidence of anomalies associated with OCs was calculated among all three groups: CL, CP and CLP. There were 434 associated anomalies in 143 cases of OCs with MCA. Most frequently affected was the musculoskeletal system (31.6%, 137 anomalies), followed by cardiovascular (21.7%, 94), but a more detailed analysis of CL and CLP cases revealed some differences in the incidence and type of associated anomalies. Cardiovascular anomalies were the most common associated anomalies (30.1%), followed by musculoskeletal (23.8%) and congenital anomalies of ear, face and neck (20.6%) in

the CL group, while the most common associated anomalies in the CLP group were musculoskeletal (32.9%), cardiovascular anomalies (22.7%) and anomalies of central nervous system (10.2%). There are also some differences in pairs of associated anomalies with OCs. The results indicate that CL and CLP could be genetically distinct entities and should be analyzed separately when possible.

#### P01.338

##### Phenotypic diversity of oral-facial-digital syndrome

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The oral-facial-digital syndromes (OFDS) are variable group of disorders characterized by malformations in oral cavity, face and digits on hands and feet. Nine different subgroups are described depending on the severity of the above mentioned anomalies as well as on the additional anomalies of the brain, kidneys, limbs, eyes and other organs. However, overlap between groups occurs frequently in described cases.

Four patients with different type of oral-facial-digital syndrome (OFS) have been described. The patients showed variable anomalies on oral structures, tongue and digits on hands and feet. Anomalies of other organs and systems were noticed, including brain (1 patient), heart (1 patient) and kidneys (1 patient). The diagnosis of OFS types I, IV, and VI (two patients) has been established. Severity of the disease varied in all of them.

Minor facial anomalies, digital malformations, as well as the existence of additional malformations of other organs enable classification of the patients in subgroups. Although there are attempts for classification according to specific criteria, the diagnosis of the subtype is not always easy and clear. Many doubtful cases have been reported. The similarity and diversity of the clinical findings of the above described cases points out the difficulty in delineation of the subtypes of OFS syndrome because of the overlapping features between them and with related syndromes.

#### P01.339

##### Randomized dose comparison of pamidronate in children with types III and IV Osteogenesis imperfecta: 3 vs 6 month cycles

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Aim: To determine whether the vertebral benefits of q3m infusion cycles can be attained on q6m cycles, with a lower cumulative dose. Methods: Twenty-seven children with types III and IV OI were randomly assigned to receive 1mg/kg/3d IV pamidronate in q3 or q6 month cycles. All patients had spine radiographs, L1-L4 DXA, and musculoskeletal and function testing.

Results: L1-L4 DEXA increased significantly after 1 year of q3m cycles, with average change in z-score =+1.41 SD, but did not improve significantly with further treatment. In the q6m group, the average change in DEXA was not significant. Repeated measures analysis of DEXA z-scores yielded a z-score rate change of 0.064 SD/m for q3 vs 0.036 SD/m for q6 group ( $p=0.13$ ). T12-L4 vertebral area and central height were determined from radiographs. Repeated measures analysis revealed significant improvement of q6m group average L1-L4 and T12-L2 vertebral height ( $p=0.05, 0.01$ ) and area ( $p=0.002, 0.006$ ). The rate of improvement of the q3m and q6m groups did not differ for L1-L4 area or height ( $p=0.52, 0.86$ ) or T12-L2 area or height ( $p=0.28, 0.77$ ). The OI children had no significant improvement in fracture incidence, manual muscle testing or BAMF motor scores in either group. Note-worthy, response to treatment was highly variable in each treatment group; improvement in vertebral area did not correlate with change in DEXA z-score.

Conclusions: Equivalent gains in vertebral height and area are obtained with q6m and q3m pamidronate cycles. For individual OI children, gain in DEXA does not correlate with extent of vertebral response.

#### P01.340

##### Osteoporosis-Pseudoglioma syndrome in two sisters

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Osteoporosis-Pseudoglioma Syndrome (OPPG) is an autosomal recessive disorder characterized by congenital blindness and severe juvenile osteoporosis leading to fragility of long bones. Affected individuals often have deformed bones due to fractures. The ophthalmologic findings may include phthisis bulbi, microphthalmia, and some vitreoretinal changes. The OPPG locus is mapped to 11q12-13 and mutations that cause OPPG were identified in the LRP5 gene. LRP5 is a member of low density lipoprotein receptors (LDLR) family and a component of the Wnt pathway. Here, we report two sisters diagnosed as OPPG who were referred to genetic service with osteogenesis imperfecta, bilateral congenital nystagmus and microphthalmia.. Their parents were phenotypically normal first cousins. The older sister was 18 years and the younger was 14 years old. They had congenital blindness and both had operations due to long bone fractures resulting in inability to walk. Physical examination findings included microphthalmia, scleral opacity and skeletal deformities especially in the lower limbs of both sisters. Tc-99m MDP whole-body scintigraphy showed deformation and asymmetry of the lower limbs and axial bones of the older sister while the younger one had normal scintigraphic findings. Dual-energy X-ray absorptiometry measurements revealed osteoporosis of both cases. According to these findings OPPG was prediagnosed and LRP5 gene mutation screening is planned.

#### P01.341

##### Overgrowth and X chromosome imbalance: Study of two unrelated females

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In humans, partial X chromosome duplications/deletions are relatively rare chromosome rearrangements, described predominantly in males with mental retardation and generally associated with phenotype anomalies. Nevertheless affected females have also been reported although most of them do not show abnormal clinical findings, probably due to skewed, preferential inactivation of dup/del(X) chromosomes, and subsequent selection against cells with an active abnormal X in carrier females. However, some females with dup/del(X) chromosomes and random X-inactivation also exhibit developmental anomalies. Therefore, females with two X chromosomes and an active duplicated/deleted segment are functionally disomic/monosomic for genes that are normally subject to X-inactivation and functionally trisomic/disomic for those genes that escape X inactivation.

Here, we report on two unrelated females with overgrowth, Sotos-like phenotype, mental retardation, normal sexual development and de novo X chromosome anomalies, both originated in the paternal X chromosome and showed random X-inactivation. Diagnosis was confirmed by array-CGH and MLPA, and completed by X-inactivation and microsatellite studies. The first case, a 14 year-old girl with epilepsy carried a 20.2 Mb Xp duplication (p11.3 → p21.3). The second, a 17 year-old female was found to have a 26 Mb Xq deletion (q24-qter) and she had idiopathic thrombocytopenic purpura (ITP). These findings indicate that a gross functional imbalance in the cells with functional disomy/monosomy due to an active dup(Xp)/del(Xq) chromosome respectively, may have caused the same effect on prenatal and postnatal growth in both patients.

#### P01.342

##### Vitamin A deficiency in a neonate with PAGOD syndrome

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PAGOD syndrome is a rare condition characterized by multiple congenital anomalies. These include pulmonary artery and lung hypoplasia, agenesis, diaphragmatic abnormalities and congenital cardiac defects. Omphalocele, various degrees of genital anomalies, cleft palate and optic nerve hypoplasia have also been described in affected patients. The etiology of this condition is still unknown. The spectrum of

birth defects is similar to developmental anomalies that are observed in an animal model with vitamin A or retinoic acid deficiency. We describe an infant with hypoplastic left heart, right pulmonary artery and lung hypoplasia, eventration of right hemidiaphragm, complete hypospadias and absent gonads. The karyotype was normal (46,XY) and FISH for SRY locus was present. Endocrine evaluations were suggestive of primary hypogonadism. Low testosterone, AMH (anti mullerian hormone) and Inhibin B values along with absence of visualized testicular tissue and Mullerian structures by imaging studies, indicated a defect in early embryogenesis. Interestingly, the level of plasma free retinol was low, consistent with severe vitamin A deficiency, supporting the hypothesis that a defect in vitamin A metabolism may have an etiological role in this syndrome with multiple congenital anomalies.

#### P01.343

#### Perrault Syndrome: Report of Four New Cases, Review and Exclusion of Candidate Genes

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Sensorineural hearing impairment and ovarian dysgenesis are the main clinical signs of the Perrault syndrome. This syndrome was first described by Perrault et al in 1951. Since this first report, about 30 cases were described. More recently, some authors have reported neurologic abnormalities in Perrault syndrome, in particular progressive cerebellar ataxia and mental retardation. We present here on three sporadic and two familial new cases of Perrault syndrome. Only two of them present with neurological defect. We analyse the clinical features of this five patients and review the published cases in order to evaluate the frequency of the neurological defect in this syndrome. Moreover, we exclude *GBG2*, *POLG* and *FOXL2* as candidate genes in Perrault syndrome.

#### P01.344

#### Susceptibility markers for PFAPA disease

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Periodic fever syndrome (PFAPA) is characterized by aphthous stomatitis, pharyngitis and cervical adenitis. The periodic fever and auto-inflammatory syndromes constitute a group of diseases characterized by repeated febrile illnesses associated with various other symptoms. Except for PFAPA, the genetic basis of each of these diseases is known. Predominant mutations in *MEFV*, *TNF1A* and *CARD15/NOD2* were analyzed in 57 children diagnosed with PFAPA.

Children with PFAPA were carefully selected based on clinical signs and symptoms. All patients were recruited at the Department of Pediatrics, Meyer Hospital of Children, Rambam-Health Care Campus during 2006-2007. Parents were invited to include their PFAPA diagnosed children in the study and to sign informed consent forms as customary. Clinical information was complemented during physicians-parents encounter and a blood sample was drawn for molecular testing. PCR and RFLPs for the predominant mutations in *MEFV*, *TNF1A* and *CARD15/NOD2* genes were performed.

The cohort consisted of 57 children with PFAPA [33 (58%) boys; 24 (42%) girls]. The mean age at diagnosis was 30.64±16.4 months, boys were diagnosed earlier than girls (26.18±13.83 and 36.41±18.32 months, respectively, p=0.05). Predominant mutations in the *MEFV* genes were found in 16 (28.1%) children, mutations in *TNF1A* were found in 3 (5.2%) children and mutations in *CARD15* also in 3 (5.2%) children. The clinical symptoms (e.g. pharyngitis, aphthous stomatitis, abdominal pain and cervical adenitis) were equally manifested between carriers and non-carriers.

In Israeli children diagnosed with PFAPA a higher frequency of mutations in *MEFV* gene were found compared to that observed in the general population.

#### P01.345

#### Analysis of the CAG repeat and A467T and W748S POLG mutations in Iranian patients With Multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune multifactorial disease that usually develops in susceptible young adults. A possible involvement of mitochondria in MS has been postulated because of a higher rate transmission of the disease from mother to child than from father to child. Also association between Leber's Hereditary Optic Neuropathy a mitochondrial disease and MS is another evidence. Fatigue is a common problem of the MS patients which is related to the energy production difficulty by the mitochondria. To investigate further the relationship between MS and mitochondria we analysed the gene encoding for polymerase G (POLG). Among the nearly 50 disease mutations in the gene for the catalytic subunit of *POLG*, The A467T mutant enzyme possesses only 4% of wild-type DNA polymerase activity.

Polymerase gene (pol G) is a two-subunit complex consisting of a 140-kDa catalytic and a 55-kDa accessory subunit (p55). The N-terminal-catalytic subunit contains a trinucleotide CAG repeat encoding a poly-glutamine tract near the amino-terminus of the protein. Expansions of similar polyglutamine-encoding CAG microsatellite repeats in other genes are known to cause neurodegenerative disorders.

Total genomic DNA was extracted from 60 idiopathic MS Patients and 40 controls. Primers were designed to amplify the 4 hot exons and CAG repeat length of the gene following by sequencing. The distribution of the *POLG* CAG repeat length in the control samples matched the distribution reported for control samples by others. The analysis of our sample shows no difference between the CAG repeat length distribution of control and MS disease samples analysis of exon 7, 8, 9, and 13 of *POLG* gene from 60 DNA samples of MS patients showed no mutation. However this does not exclude the association of the gene in MS but it needs more investigation. Also it is necessary to test the other exons of the gene.

#### P01.346

#### *TCF4* deletions in Pitt-Hopkins syndrome

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Background: Pitt-Hopkins syndrome (PHS) is a syndromic mental retardation disorder marked by hyperventilation episodes and characteristic dysmorphisms (large beaked nose, wide mouth, fleshy lips, and clubbed fingertips). PHS has been shown to be caused by de novo heterozygous mutations of the *TCF4* gene, located in 18q21. To expand the phenotypic spectrum of mutations and deletions of the *TCF4* gene, we studied 30 hitherto unexplored patients whose phenotype overlapped with PHS.

Methods: The *TCF4* gene was analysed by QM-PCR, followed by sequencing. Large deletions were characterised by CGH array. All patients were karyotyped.

Results: In three patients, 18q21.1-q22.2 deletions were revealed by

karyotyping. QMF-PCR permitted to detect one large deletion and six small deletions generating premature stop codons.

The patients with small deletions had characteristic dysmorphic features. All patients had severe developmental delay, late (after 5 years) or absent walking, no speech, and microcephaly. We found a high incidence of myopia in all patients who had undergone eye testing. We frequently observed a happy disposition, stereotypic movements and strabismus. In contrast, hyperventilation, epilepsy, constipation were inconstant, and none of them had Hirschsprung disease or visceral malformation.

**Conclusion:** We report 10 novel *TCF4* deletions, in patients whose phenotype strongly overlapped with PHS. However, we observed phenotypic variations between the small deletions and the large genomic deletions, which may lay the basis for further genotype-phenotype correlations at the *TCF4* locus. Owing to the large number of deletions and microdeletions/insertions in *TCF4* we would address to start the molecular study by a QMF-PCR analysis.

#### P01.347

##### Medical and auxological outcome in seventy 2-year-old singletons born after embryo biopsy applied in preimplantation genetic diagnosis or preimplantation genetic screening

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**Introduction:** Limited data are available on the growth and clinical outcome of children born after embryo biopsy. Embryo biopsy is an invasive procedure to perform preimplantation genetic diagnosis (PGD) or screening (PGS). The objective was to determine if embryo biopsy might cause prenatal and/or postnatal growth restriction and induce congenital malformations.

**Materials and methods:** In this study we compared growth and physical findings between seventy 2- year -old singletons born after PGD/PGS compared to intracytoplasmatic sperm injection (ICSI) and spontaneous conception (SC). Children were matched for gender, maternal educational level, mother tongue and birth order.

**Results:** No differences were found regarding weight, height and head-circumference standard deviation scores at birth and at age two years. Body Mass Index standard deviation score in PGD/PGS children at age two years was lower compared to SC children ( $p = 0.05$ ). PGD/PGS children were more frequently born after caesarian section but had no more congenital malformations, hospital admissions and surgical interventions. Growth parameters within the PGD/PGS group of children born after biopsy of one or two blastomeres were comparable.

**Conclusions:** Singleton children born after embryo biopsy applied in PGD/PGS present a similar prenatal and early postnatal linear growth compared to ICSI children and SC children. PGD/PGS singletons appear not at higher risk for congenital malformations and surgical interventions. Body Mass Index standard deviation score was slightly lower in PGD/PGS children compared to SC children. There are no observable detrimental effects of the PGD/PGS procedure on children during the first years of life.

#### P01.348

##### Primary hyperoxaluria in Italy

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Primary Hyperoxaluria (PH) is a rare autosomal recessive disease with impaired hepatic detoxification of glyoxylate, due to AGT (PHI) or GRHPR (PHII) enzyme deficiency. Oxalate overproduction in turn causes nephrolithiasis, end-stage renal failure, systemic oxalosis and multi-tissue damage. In responsive subjects an early biochemical and genetic diagnosis can address to treatment with vitamin B6 (the AGT cofactor). In many cases liver or combined liver/kidney transplant is necessary to correct the metabolic defect.

In a cohort of 47 PHI and 2 PHII Italian patients we identified the pathogenic mutation of 90/94 PHI and 4/4 PHII alleles (96% mutation detection)

using DHPLC and DNA sequencing.

Age at presentation varied from 1 month to 49 years (median 6 y). AGT residual activity in liver biopsies is available for 34 subjects. In 33 it was possible to define B6 responsiveness by comparing plasma oxalate before and after oral supplementation.

23 different mutations were found (6 unpublished); missense mutations affect evolutionary conserved residues and are absent in 160 chromosomes of healthy ethnically matched controls.

Genotype-phenotype correlations show an important role of non-genetic factors as diet or delayed diagnosis, and confirm in our population that the most frequent mutation G170R, causing mitochondrial mistargeting, is associated with a mild phenotype (residual enzymatic activity, late onset and responsiveness to B6). For missense (G116R) and *insdel inframe* (c.283dupGAG) mutations we presume an *antimorphic* effect as they were found only in patients with a severe phenotype despite the presence of a mild mutation on the other allele.

#### P01.349

##### Wiedemann-Rautenstrauch syndrome:case report

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Wiedemann-Rautenstrauch syndrome or neonatal progeroid syndrome is a rare autosomal recessive disorder, with few published case reports. WRS is characterized by progeroid appearance at birth, lipoatrophy, slow growth .

We report a 8 months old female, the fourth child of a healthy nonconsanguineous couple. The pregnancy was unremarkable. The birth was at term with prenatal hypoplasia and aged signs present at birth. (BW 2500 g, BL 47 cm). At age of 8 months old progeroid appearance became more pronounced. The patient showed growth delay (W=3,9kg; L=60cm,), muscle hypotrophy, psychomotor retardation and typical progeroid features; senile-appearing triangular face, beak-shaped nose, microstomia, micrognathia, congenital incisors, hydrocephaloid cranium, widened fontanelles and sutures, prominent veins on scalp, hypotrichosis, relatively large fingers and toes, wrinkled skin, lipoatrophy with scleroderma-like changes of skin on the buttocks. She had feeding problems.

Congenital cataract and glaucoma were revealed. Ultrasound examination of brain, heart and abdomen did not reveal any abnormalities. Chromosomal and biochemical analyses were normal. Clinical features of our patient were compared with published data of WR syndrome, other premature aging syndromes, nonclassified progeroid conditions. We have established "neonatal progeroid Wiedemann-Rautenstrauch syndrome" based on association of characteristic aging appearance present at birth, failure to thrive, deficient growth and development, hypotrichosis, signs of generalized lipoatrophy, scleroderma-like changes of skin on the buttocks and congenital incisors. The parents were informed about the genetic risk of recurrence.

#### P01.350

##### A case report of proteus syndrome

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Proteus Syndrome (PS) was initially described in 1979 by Cohen and Hayden and assigned its name several years later in 1983 by Wiedemann. This is a relatively recently delineated syndrome, probably because it is so rare and because it overlaps with a number of other asymmetric overgrowth syndromes.

The disorder primarily manifests as postnatal overgrowth, with irregular, distorting and progressive overgrowth that can include many tissues essentially connective tissue, bone, skin, central nervous system, and the eye.

The overgrowth of PS is progressive and can be difficult to manage. It can cause severe orthopaedic complications.

One of the most common complications in patients with PS is deep venous thrombosis and pulmonary embolism, which can cause premature death.

The management of this syndrome requires knowledge of the wide array of manifestations and complications and a multidisciplinary approach. Patients with PS have an increased risk of developing tumours.

The cause of this disorder is unknown. All cases have been sporadic events in the otherwise normal families. It is most likely caused by a somatic mutation of a gene that is lethal when occurring in the non-mosaic state.

We report on a sporadic case of two years old boy in which Proteus Syndrome is diagnosis because of a large haemangioma in the right hemi chest and the upper limb. He has also a macrodactyly of the left thumb, hemihypertrophy. He has also a multiple soft subcutaneous swellings over the chest and the abdomen.

#### P01.351

#### Identification of *PSEN1Δ9* mutation in mexican families with spastic paraparesis and presenil dementia

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Spastic paraparesis and early-onset dementia (also known as early-onset familial Alzheimer disease-3) is rare, and 26 families have been worldwide described; in most of them several mutations in *PSEN1* included point mutations (exons 3, 4, 7, 8 y 12), insertions (exon 3) and deletions (exons 4, 9, 12) that have been identified. Our aim is to detect the *PSEN1Δ9* mutation related with high frequency and severity. In Mexico there are not familial reports with this disease, therefore it is unknown if previously described mutations are present in these families.

In this study we described eight families with clinical signs that correlated with spastic paraparesis and early-onset dementia. They showed an autosomal dominant inheritance with variable expressivity. Affected subjects per family are 2 to 6 with mean onset age of 45.1 years and sex ratio of 1/0.76.

Molecular analysis demonstrated a *PSEN1Δ9* mutation in eight affected subjects and in two non-affected (33 and 36 years old), 34.78% and 6.06% of total amount respectively. At least it was identified in one affected subject out of six studied families. It was not possible to conclude that *PSEN1Δ9* mutation itself explains the presence of this phenotype in Mexican families.

#### P01.352

#### Primary pulmonary vein stenosis and lymphatic anomalies: a new syndrome?

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Primary pulmonary vein stenosis is a rare congenital vascular malformation with a high mortality rate. It is characterized by obstruction of one or multiple pulmonary veins. Most patients present in the first year(s) of life with failure to thrive, dyspnoea, and recurrent pneumonias. Later pulmonary hypertension, pulmonary oedema and haemoptysis develop. Most patients die during infancy since there are no adequate therapeutic options. So far only sporadic cases have been described. The aetiology of the disease is unknown and many possible underlying pathologic mechanisms have been described.

We present a consanguineous Turkish family with four affected siblings (three males and one female) of healthy parents. The first child died at 16 months due to progressive pulmonary vein stenosis. The following two sibs presented prenatally with cystic hygroma and mild skin oedema. Chromosome analysis was normal. At birth cardiac evaluation was normal but both developed severe and progressive stenosis of the pulmonary veins in the following months of life. Both died before the age of 8 months due to restenosis of the pulmonary veins after surgical intervention. The fourth pregnancy was terminated due to cystic hygroma and severe hydrops.

These data provide evidence for an autosomal recessive form of primary pulmonary vein stenosis associated with lymphatic anomalies and hydrops. We are performing genome wide linkage studies in this

family to locate the gene for primary pulmonary vein stenosis. This syndrome might be underdiagnosed due to post- and prenatal lethality.

#### P01.353

#### Quadrupedal Locomotion and Cerebellar Hypoplasia Caused by Mutations in the Very Low Density Lipoprotein Receptor (VLDLR) Gene

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The cerebellum is the primary motor coordination centre of the central nervous system. Lesions or congenital defects of the cerebellum cause incoordination of the muscles resulting in irregular gait and falling. Recently, we reported a large family with cerebellum hypoplasia and quadrupedal locomotion as a recessive trait, which we mapped to chromosome 17p13.

We identified one additional family with the same condition and mapped the underlying gene to a 14-centimorgan interval on chromosome 9ptel using a genome wide linkage approach. Sequencing of candidate genes identified a homozygous frameshift mutation in the Very Low Density Lipoprotein Receptor (VLDLR) gene in all affected individuals. The association of cerebellar hypoplasia with mutations in VLDLR has been reported previously in the Hutterite population and in a family from Iran. However, quadrupedal locomotion was never observed indicating that environmental factors play a major role in the pathogenesis of this form of locomotion.

#### P01.354

#### Old Iranian Scientists conception about reproduction and Genetics were almost similar to Modern Facts

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The oldest scientific hypothesis in this regards is attributed to Hippocrates, which denotes, that "man's water (semen) is an extract from the whole body and contains all the characteristics of the man".

Aristotle believed that the semen originated from blood and gave life to the clotted menstrual blood. In general, the scientists believed that all characteristics were inherited only from the father. This belief persisted for about 2000 years, until William Harvey, showed that there were no traces of blood clots in the uterus of the pregnant hunted gazelles.

C.E. Wolf in the late 17<sup>th</sup> century showed that the embryo is a product of the fertilization of ovum by spermatozoid. It is surprising that based on the Zoroastrian law the right of women and men were equal in all respects.

The Zoroastrian priests, would pass, this important position to their offspring regardless of the sex. Eight hundred years before Wolfs' discovery the sage Ferdowsi describes the royal characteristic of Kaykhusrow, the grand son of the kings Kaykavos and Afrasyab: "He, the pure bred, has inherited from two races".

More interesting, in the book written in the first half of the thirteenth century, characteristics of the spermatozoid have been described in an appealing way almost same as what we know today. Despite this brilliant history, there was a big gap in this field between our country and the developed world.

In spite of a long standstill, recently, we have moved to the front line with our outstanding advancements in diagnosis, prevention and treatment of hereditary diseases, particularly application of embryonic cells in production and restoration of body organs and cloning of "Royana", which with all rewarding advancements, have placed our country among pioneers and leaders of this caravan.

#### P01.355

#### Mitochondriopathy presenting with immune disorder

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Due to ubiquitous nature of oxidative phosphorylation and dual genetic origin of respiratory chain enzymes (nuclear and mitochondrial DNA) their deficiencies can produce any symptom in any organ. However, the involvement of the immune system in mitochondriopathies is rare. We report a girl aged 20 months with combined respiratory chain defect and immunologic impairment that includes T-cell immunodeficiency and autoimmune reactions. Main clinical findings were generalized hypotonia, rotatory nystagmus, failure to thrive and respiratory deficiency due to persistent lung infections. In trachea a large spectrum of bacteria and fungi (mostly Aspergillus fumigatus) has been detected. Cytomegalovirus infection was permanent with up to 240,000 copies of CMV DNA/ml of blood despite one year ganciclovir treatment. She had crises with fever, tachycardia and facial blushing. Laboratory findings pointed to autoimmune processes (positive anticardiolipin antibodies, positive direct and indirect Coombs test and positive antiplatelet antibodies, hypergammaglobulinemia) and immunodeficiency. The clinical impression of an immunodeficiency was supported by repeatedly increased CD4/CD8 ratio (6.3; normal 0.97-2.3) with significant decrease of CD8 T cells (9%). Flow cytometric analysis of intracellular staining of IFN-gamma and IL-4 in CD4 and CD8 T cells showed three times very low IFN-gamma in CD8 T cells (0.5-1%; reference range 2-7%).

The severely reduced activity of respiratory chain complexes I (3.0 U/gNCP; normal 15.8-42.8) and IV (53.6 U/gNCP; normal 112-351) in skeletal muscle suggested mitochondrial etiology. Sequencing of the mtDNA tRNA genes did not reveal pathogenic mutations and mtDNA depletion was excluded by real-time PCR suggesting a mitochondrial translation defect.

#### P01.356

#### Horizontal gaze palsy with progressive scoliosis can result from new missens mutation in ROBO3 gene in Russian family

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Horizontal gaze palsy with progressive scoliosis (HGPPS) is an autosomal recessive disorder characterized by congenital absence of horizontal gaze, progressive scoliosis, and failure of the corticospinal and somatosensory axon tracts to decussate in the medulla. The reasons of disease are various homozygous or compound heterozygous mutations in the axon guidance molecule ROBO3.

Two sisters seven and ten years old from Armenian family with short stature non-proportional because of spine deformity were examined by us. Both of them has progressive scoliosis (the oldest sister has right-sided scoliosis and younger has left-sided one). Now both girls have severe thorakolumbar scoliosis. Two girls have congenital external ophthalmoplegia, gorizontal gaze palsy, which at birth onset.

DNA was extracted from a blood sample from each participant using a standard protocol, and the coding exons of ROBO3 were amplified and sequenced.

Two sibs with horizontal progressive external ophthalmoplegia unique and scoliosis have c.290G>C (Trp97Ser) mutation in homozygous state in ROBO3 gene. Each of their parents was the heterozygotic carrier of this mutation. This mutation was not previously described.

#### P01.357

#### An unusual case of sirenomelia and a review of the casuistic of the Perinatal Genetic Program, UNICAMP, Brazil

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Sirenomelia sequence is a rare and etiologically unknown anomaly, which is considered as a primary defect of the blastogenesis. The purposes of this presentation is, first, reporting a case of sirenomelia associated with a unique constellation of malformations, and, second, exploring the casuistic of this anomaly in the Perinatal Genetic Program during a twenty years period. The case reported is the product of the first gestation of young and non consanguineous parents. Family history was unremarkable. Gestational period was also uneventful, except for prenatal ultrasonographic evaluation that revealed bilateral renal agenesis, absence of the left lower limb and the right fibula, and

vertebra malformations. At birth, besides the findings of sirenomelia sequence with footless, the fetus also presented bilateral anotia, an unusual cleft palate, and also an unusual phallic structure on the genital region. The review of 7 cases occurring at the same hospital showed a high prevalence (1.2 cases per 10,000 births) of sirenomelia, probably, due to the prenatal diagnosis. All are single cases. The maternal diseases referred by these respective mothers were hypothyroidism, depression and arterial hypertension. The main medicines used by them were l-thyroxine, fluoxetine, captopril, and amphetamines. Although the sonographic evaluation performed at the first trimester has been made in two cases, sirenomelia was never referred at prenatal time. In conclusion, besides the case with a rare pattern of sirenomelia associated with other no related defects, the review of seven new cases shows the inability of sonographers to make diagnosis of sirenomelia.

#### P01.358

#### Sirenomelia sequence: clinical, radiological and postmortem findings

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Sirenomelia is a Greco-Roman mythical creature with the tail of a fish and a human upper body. Similarly, Sirenomelia Sequence is characterised by fusion and reduction of lower extremities; renal and sacral agenesis; imperforate anus; absence of rectum and bladder. It is indeed an "orphan" malformation since it is not even recognised as a separate entity in the OMIM catalogue. Here, we report a total of five cases, including a pair of twin sibs, who were classified according to Stocker classification as type I, VI and VII (N=1 each) and type II (N=2). All patients had a single umbilical artery; imperforate anus, oligo hydranmion; Potter facies; congenital heart diseases; gastrointestinal and genitourinary abnormalities. One of the twins had full blown expression of Sirenomelia, whereas the other had imperforate anus only (Clinical Dysmorphology 2000; 9:227). Maternal diabetes was associated with Sirenomelia in one patient (Turkish Journal of Pediatrics 1996; 38: 393). The earliest age of prenatal diagnosis was fourteen weeks based on the observation of severe oligohydramnion and a single lower extremity at ultrasonography. Postmortem findings of this case revealed a blind end of the abdominal aorta, a single femur and hypoplastic fused tibiae, renal agenesis, a dysplastic kidney, a hypoplastic bladder, genital organ agenesis, colon abnormalities, hypoplasia of the cerebellum and the posterior fossa. Severe maternal B12 and Folic Acid deficiency were present in this case. These etiological factors have not been reported previously although various teratogenic events have frequently been associated with Sirenomelia.

#### P01.359

#### Sirenomelia with a de novo balanced translocation 46,XX,t(X;16)(p11.3;p12.3)

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Sirenomelia is a rare developmental abnormality, characterized by the fused lower limbs, and abnormalities in the caudal abdomen and pelvis. Most cases are sporadic and specific causes and early pathogenetic events leading to sirenomelia are unknown. We report a singleton female fetus with sirenomelia with 46,XX t(X;16)(p11.3;p12.3)dn. The pregnancy was electively terminated at 21 weeks gestation after prenatal ultrasound examination. The birth weight was 294g, and length 25.5cm. The external genitalia was ambiguous and anal atresia was noted. At autopsy, anatomical findings included absence of bilateral kidney and bladder, hypoplastic or absence of renal artery and inferior mesenteric artery. Unfortunately the results of karyotype were revealed after autopsy. The cytogenetic analysis of breakpoints was so limited. FISH experiments with BACs were employed for narrowing of the breakpoint regions. On chromosome 16, the breakpoint was mapped between RP11-347K10 (16p12.3, 17.55Mb from pter) and RP11-167K14 (16p12.2, 20.87Mb from pter). On chromosome X, the breakpoint was mapped between RP11-245M24 (Xp11.3, 45.36Mb from pter) and RP11-416B14 (Xp11.23, 48.46 Mb from pter). Abnormal phenotype, present in balanced translocation, was caused by deletion or breakage of dosage-sensitive genes of breakpoint, or disruption of an imprinted gene. In addition, cases with balanced translocations

were assumed to be at risk for UPD. To date, only one case with abnormal karyotype involving maternal UPD16 was reported as body stalk anomaly [Chan et al., 2000]. Although the parental origin of normal 16 and der(16) remained to be determined, this case will provide insight into consideration of pathogenetic mechanism of sirenomelia.

#### P01.360

#### Hereditary spastic paraplegia, type 4, in Russian families

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Hereditary spastic paraplegia (HSP), type 4, or SPG4, caused by various mutations in spastin gene (*SPAST*) is the most common disorder in a heterogeneous group of autosomal dominant HSP's. We performed a search of *SPAST* mutations by routine methods (SSCP and subsequent direct sequencing of fragments with modified electrophoretic mobility) in a sample of 26 families with autosomal dominant HSP from different Russian regions. In six families, five of Russian and one of Tatar ethnicity, different *SPAST* mutations were detected. Three of them, Arg431Stop, 839-840 delAG, and Asn386Ser, were already reported; the remaining three, Asp555Tyr, 1107A>G, and Asn184Thr, were novel. One more large family showed a linkage to SPG4 locus (lod score 1.51) but mutation was not found. This may be due to atypical *SPAST* mutations (partial deletions etc) undetectable by routine methods of DNA analysis. Including this family, the proportion of SPG4 in the sample is 27%, which is less than average world data (40-50%). Most of our patients presented relatively late-onset "uncomplicated" HSP, which is typical for SPG4. Though, in some families additional features were reported, epilepsy among them. One of our patients had very early-onset HSP and concomitant epilepsy while his mother had typical SPG4 presentation. All pedigrees showed complete penetrance though some patients, women particularly, had mild signs of the disease, even late in life. Another relatively frequent autosomal dominant HSP is SPG3 caused by mutations of atlastin gene (6-10%). We plan to perform a search of atlastin mutation in families without *SPAST* mutations.

#### P01.361

#### Glaucoma, short stature, mental retardation and ischemic stroke: A new autosomal-recessive syndrome mapping to Chromosome 20q11-12

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We report on a family with multiple affected siblings of both sexes presenting with a novel combination of anomalies including cerebrovascular insults. The four affected probands were born to consanguineous Turkish parents, there are six non-affected siblings. All four probands have a short stature. The eldest male had congenital left sided glaucoma, presented with a stroke at the age of 6 months and showed a severe developmental delay. He died at 40 years of age. The second proband suffered from right-sided congenital glaucoma and recurrent strokes starting at the age of one year. He was wheel-chair dependent and developed multiple joint contractions. He died of an intracranial hemorrhage at the age of 28 years. The third proband had bilateral congenital glaucoma. She is only mildly developmentally retarded, but also developed multiple joint contractions. The youngest proband also suffers from congenital left sided glaucoma and severe developmental delay. She was never able to walk and showed precocious puberty. At the age of 14 years, she had an ischemic stroke of the left arteria cerebri media. MRI angiography showed total occlusion of the internal carotid arteries bilaterally and of the left arteria cerebri media. Homozygosity mapping in this family, using a high density SNP array, identified a candidate locus of 7.3cM on chromosome 20q11-12. The familial association of congenital glaucoma, mental retardation, short stature and ischemic stroke is a hitherto unreported entity. This autosomal-recessive syndrome is mapping to chromosome 20q11-12.

#### P01.362

#### Screening of telomeric regions by MLPA in more than 470 patients with mental retardation and fetuses with malformations

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Cryptic telomeric rearrangements are frequent causes of mental retardation and/or malformation syndromes. We used for more than 2 years, a systematic screening of all subtelomeric regions using MLPA with two commercial kits from MRC Holland.

We studied more than 470 patients, including 44 fetuses with malformations. Except for these fetuses, all the patients had mental retardation, associated for most of them with some malformation or dysmorphic features. In every cases, but four, standard karyotype analysis was normal.

Only one fetus (2,5 %) is probably positive. This case is likely a 15q deletion, but FISH analysis was not possible to confirm it.

Among our postnatal cases, 15 anomalies, not suspected on karyotype analysis were confirmed (3,5%) and 4 suspected anomalies were further analyzed.

In addition some anomalies with only one MLPA kit were identified in an unaffected parent and therefore considered as polymorphism. On the opposite, true anomalies were sometimes detected with only one MLPA kit.

Unexpectedly, 5 anomalies implied M9p, including 3 «pure» subtelomeric monosomies. One case was familial, inherited from the mother (with border-line mental retardation).

In conclusion, MLPA technique for telomeric screening is interesting and not very expensive. Furthermore, some of the anomalies we detected here, were almost «non-syndromic», favoring a non-specific all-telomeres screening approach in mental retardation.

At last, further molecular analysis, including CGH-array, of our M9pter cases is in progress to refine phenotype/genotype correlations.

#### P01.363

#### Testicular dysgenesis syndrome: more common after assisted reproduction?

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Recent reports have demonstrated a decline in human male reproduction health: increasing prevalence of cryptorchidism, hypospadias, poor semen quality, rising incidence of testis cancer and growing demand for assisted reproduction. It is supposed that these are symptoms of one underlying entity, the testicular dysgenesis syndrome (TDS). The rapid rise in the prevalence of TDS may be linked to endocrine disrupters affecting genetically susceptible individuals. Genetic aberrations or polymorphisms may predispose to augmented effects by environmental factors. Meta-analysis of in vitro fertilization (IVF) studies revealed that hypospadias is more common after assisted reproduction (AR). In Hungary about 2000 infants are born following IVF yearly. In our study we investigated the incidence of congenital abnormalities after AR. At the Department of Obstetric and Gynaecology, Medical School, University of Pécs 712 newborn infants were born after IVF between January 1<sup>st</sup>, 1999 and December 31<sup>th</sup>, 2006, 319 singletons and 178 sets of twins. The mean gestational age was 35.6±2.1 weeks, the mean birthweight 2520±630 grams. The incidence of congenital abnormalities in infants born after conventional IVF was compared to newborns born after intracytoplasmatic sperm injection (ICSI). In regard to major congenital abnormalities we did not find any significant differences between the two groups (5.8% vs 3.2%, p=0.056). Infants born after ICSI significantly more often suffered from minor anomalies [p=0.004, OR:1,9478 (95%CI:1.1916-3.1841)], especially from genital abnormalities, hypospadias, cryptorchidism, hydrocele testis [p=0.031, OR:2,48 (95% CI:0.9949-6.1594)]. Further investigations will be needed to confirm the supposition that children born after IVF/ICSI are at higher risk to develop TDS.

**P01.364****Familial hypodontia associated with taurodontism****E. Severin, C. Albu, D. Stanciu, D. F. Albu;***"Carol Davila" Univ Med Pharm, Bucharest, Romania.*

Tooth agenesis and taurodontism are developmental dental anomalies. Some studies support the hypothesis that isolated taurodontism is associated with tooth agenesis, as it shows a higher prevalence in families with missing teeth than in general population, and, therefore, may share a common genetic cause. We report a case of a mother and her two daughters affected of tooth agenesis. Mother and daughters exhibited different clinical manifestation of missing teeth. Simultaneous occurrence of taurodontism and tooth agenesis was detected only in mother. Evaluation included clinical, radiographic and genetic examinations. The affected members of the family did not show features of syndrome tooth agenesis. No specific correlation between missing teeth and taurodontism was noted.

**P01.365****A de novo microdeletion of 774 kb including the entire TP53 gene - Li-Fraumeni-plus as a contiguous gene syndrome?****T. Schwarzenbauer<sup>1</sup>, A. C. Obenauf<sup>1</sup>, A. Langmann<sup>2</sup>, U. Gruber-Sedlmayr<sup>3</sup>, K. Wagner<sup>1</sup>, M. R. Speicher<sup>1</sup>, P. M. Kroisel<sup>1</sup>;**<sup>1</sup>*Institute of Human Genetics/Medical University of Graz, Graz, Austria, <sup>2</sup>Department of Ophthalmology/Medical University of Graz, Graz, Austria, <sup>3</sup>Department of Pediatrics/Medical University of Graz, Graz, Austria.*

Li-Fraumeni syndrome (LFS) is a rare genetic disorder usually characterized by familial occurrence of various malignancies with early onset. LFS is clinically and genetically heterogeneous. The inheritance of LFS follows an autosomal dominant mode with high penetrance and mutation rate in germ cells is very low. In most cases LFS is due to a constitutional mutation of one copy of the *TP53* tumor suppressor gene in affected family members and somatic mutations in the second copy of that gene in the process of malignant transformation. The *TP53* gene at chromosome 17p13.1 is the most frequent target for genetic alterations in human cancer.

Here we describe the first case of a de novo constitutional chromosomal microdeletion including the entire *TP53* gene as well as several closely linked genes. The phenotype of the patient is striking and includes severe psychomotor retardation, a Dandy-Walker anomaly, Leber congenital amaurosis (LCA), seizures and dysmorphic features. Chromosomal breakpoints (BP) were mapped by high resolution array comparative genome hybridization (aCGH) followed by quantitative real type PCR analysis down to base pair level. The distal BP disrupts the *ACADVL* gene and the proximal BP is located about 6.5 kb upstream of the *GUCY2D* gene disrupting its upstream regulatory sequences. SNP analysis revealed monoallelic expression of *GUCY2D* which would explain the LCA of the patient. More than 40 genes have been mapped to the 774 kb deletion interval including the *FXR2* gene which is an autosomal homolog of the *FMR1* gene.

**P01.366****Hypogonadism and Novel Craniofacial Findings in a Case of Treacher-Collins Syndrome with a Pathogenic Mutation and a Missense Variant in the TCOF1 Gene****C. Li;***McMaster University Medical Centre, Hamilton, ON, Canada.*

A severe case of Treacher-Collins syndrome with novel findings in a newborn boy is presented here. Craniofacial features include bilateral atretic internal auditory canals, macrocephaly with remnant lobules of the external ears, absence of external auditory canals, missing ossicles with hypoplastic middle ear cavities. The nasal cavities were filled with a soft tissue mass that may represent an encephalocele. The eye cavities were extremely underdeveloped. The inferior wall was absent, the zygomatic arches were absent. There was severe micrognathia and concurrent narrowing of the pharyngeal space, resulting in markedly narrowed upper airway. Moreover, the baby was found to have widely spaced nipples, hypoplastic scrotum, bilateral cryptorchidism and micropenis. Molecular testing revealed a pathogenic mutation and a novel missense change. The mother carries the same missense change and is clinically and radiographically normal. The potential significance of these findings is discussed.

**P01.367****Coexistence of Unverricht-Lundborg disease and congenital deafness in one Serbian family****M. Kecmanovic<sup>1</sup>, A. Ristic<sup>2</sup>, D. Sokić<sup>2</sup>, M. Keckarevic-Markovic<sup>1</sup>, D. Keckarevic<sup>1</sup>, S. Romac<sup>1</sup>;**<sup>1</sup>*Faculty of Biology, Belgrade, Serbia, <sup>2</sup>Institute of Neurology, Belgrade, Serbia.* Unverricht-Lundborg disease (ULD) is an autosomal recessive disorder caused by mutations in cystatin B (*CSTB*) gene located on chromosome 21q22.3. Majority of the ULD chromosomes carry expansion of dodecamer repeats in promoter region of the *CSTB* gene. The location of *TMPRSS3* gene, in which mutations cause nonsyndromic recessive deafness, is in proximity of *CSTB* gene, on chromosome 21q22.3. Here we present a complex syndrome in one Serbian family in which three out of four siblings of healthy parents have inborn deafness. Action and stimulus sensitive myoclonus appeared in two individuals (II-1 and II-3) in 12-13 year of life (individual II-1 was not available for this analysis). Except congenital deafness, individual II-2 is otherwise healthy, while the individual II-4 has none of two mentioned disorders. Molecular diagnostics confirmed suspected ULD in sibling with progressive myoclonic epilepsy and innate deafness, while the sibling with isolated inborn deafness was heterozygote for expansion in the *CSTB*. One could exclude coexistence of *CSTB* and *TMPRSS3* mutations in this pedigree due to assumed joint transmission. For that reason we performed haplotype analysis, genotyping seven microsatellites flanking *TMPRSS3* and *CSTB*. Haplotype analysis showed that absence of homozygous expansion in the individual II-2 is due to a recombination event that occurred ahead of *CSTB*, what was the strong indication that homozygous mutation in the *TMPRSS3* is responsible for deafness. Following sequencing of the *TMPRSS3* revealed 207delC mutation in fourth exon.

To our best knowledge this is the first genetically confirmed case of coexistence of mentioned two mutations.

**P01.368****Central Nervous System involvement in patients with vascular cutaneous anomalies****C. Mellado, M. Sahin;***Neurology Department, Children's Hospital Boston, Harvard Medical School, Boston, MA, United States.*

Vascular cutaneous anomalies could be related to an underlying systemic disease and/or involve other systems. One of these could be the Central Neural System, leading to a variety of neurological complications. The purpose of this study is to describe brain anomalies among individuals affected with some of these syndromes, including PHACE, Macrocephaly and Cutis Marmorata Telangiectasia Congenita (M-CMTC), Sturge Weber (SW) and Klippel Trenaunay (KT).

We reviewed 216 clinical records of patients seen at Children's Hospital Boston, between 2000 and 2007, with the diagnoses mentioned above. Brain imaging information was obtained. In 11/11 PHACE syndrome cases, beside vascular anomalies, neuroimaging showed hypoplastic corpus callosum (2), cerebellar involvement (3), asymmetric ventricles (1), retro cerebellar cyst (1), moyamoya phenomenon (1). 8/10 M-CMTC cases have images, showing hemimegalencephaly (4), Chiari I malformation (4), asymmetric/large ventricles (4), white matter changes (3), cortical dysplasia (2), periventricular heterotopias (1), brain atrophy (1), periventricular leukomalacia (1). 36/55 SW cases have images, that showed leptomeningeal enhancement in almost all cases, normal brain structures (5), cerebral atrophy (18), prominent choroid plexus (17), calcifications (5), venous anomalies (5), white matter anomaly (5), Chiari I malformation (1), asymmetric ventricles (1), mega cisterna magna (1). 20/140 KT cases have images that showed normal brain images (9), white matter changes (5), Chiari I malformation (3), calcifications (2), venous anomalies (2), asymmetric brain hemispheres (1), cerebral atrophy (1), thick corpus callosum (1).

Brain imaging is important in these syndromes to define the pathologic entity, help in the diagnosis, management and prognosis in children at risk.

**P01.369****Three novel mutations in FRMD7 in X-linked congenital motor nystagmus in Russian families**

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X-linked congenital motor nystagmus is a common inherited oculomotor disorder characterized by repetitive uncontrollable ocular oscillations, unassociated with a number of ocular or neurological diseases with onset typically at birth. The loci for X-linked CMN have been mapped to Xp11.3-p11.4 and Xq26-q27 (NYS1). Three more loci have been described for autosomal-recessive and autosomal-dominant form of CMN without any gene identification. The molecular characterization of NYS1 has been solved by Tarpey et al., who identified mutations in *FRMD7*, a gene with unknown function.

The aim of our research was searching for *FRMD7* mutations in three Russian families with X-linked CMN, who had already shown the linkage with Xq26-q27 locus. Sequencing analysis of all exons and intron-exon junctions of *FRMD7* was performed in affected males from these families. We identified three novel, previously unreported mutations in *FRMD7*: missense mutation c. 47T>C (Phe16Ser), nonsense mutation c. 1524G>A (Trp508Stop) and small deletion c. 1492delT. Thus the results of our study confirm the role of *FRMD7* in the pathogenesis of X-linked CMN.

**P01.370****Diagnostic and prognostic implications of nuclear and mitochondrial mutations in couples opting for ART**

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Microdeletion on the long arm of the Y chromosome and mutations in mitochondrial genes regulating oxidative phosphorylation may severely impair spermatogenesis. Thus it was planned to analyze cases (n = 580) with idiopathic male infertility opted for ART by cytogenetic, Yq microdeletion and mitochondrial mutation analysis.

In cytogenetically normal cases microdeletion analysis was done using EAA guidelines. Mitochondrial genome was amplified and sequenced using a set of 24 primers in 33 OAT cases. The mutations were compared in blood and sperm DNA. 8.2% and 10.1% cases harboured Yq microdeletion in blood and semen respectively. Results of mitochondrial mutation analysis showed G to A transition in ND4 gene at nucleotide position 11719 in sperm DNA of 19 cases and only in 14 cases from blood DNA. Though this is a non-synonymous change, the amino acid remains the same. The polymorphism A750G, A4769G and A8860G was found in all the semen as well blood DNA of the cases but only in 12 controls. A750G, A4769G are non-synonymous changes but A8860G polymorphism in ATPase 6 gene changes amino acid threonine to alanine.

As majority of these infertile couples opt for ART/ICSI, it is very important to distinguish cases with Yq microdeletions and mitochondrial mutations as former are iatrogenically transmitted to the offspring through these techniques. Thus a thorough genetic analysis is a must in all infertile couples to provide comprehensive counseling and most adapted therapeutics.

**P02. Cytogenetics****P02.001****Screening for subtelomeric aberrations using multiplex ligation dependent probe amplification (MLPA)**

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Subtelomeric rearrangements are believed to be a common cause of mental retardation. New molecular techniques allow their identification, their frequency and significance are still unknown.

METHODS: We screened 30 patients with idiopathic mental retardation and/or phenotypic malformations making use of the multiplex ligation-dependent probe amplification (MLPA, SALSA kits P036D and P070). The parents of those patients with subtelomeric aberrations were also tested for the origin (see table 1). All rearrangements were confirmed by FISH or by the SALSA MLPA KIT P096-MR2.

RESULTS: We identified a total of 7 subtelomeric aberrations (23.3%). Four were deletions, two were duplications and one case showed one deletion and one duplication (See table 1).

Table 1.

Case	MLPA aberration	Confirmation	Origin
1	del 4p	+ Wolf-Hirschhorn with P096 KIT - FISH with WHSC1 probe	de novo
2	del 20p	FISH in process	unknown
3	del 4p	+ Wolf-Hirschhorn with P096 KIT + FISH with WHSC1 probe	de novo
4	del 1p	+ FISH 1p36	de novo
5	dup Xq/Yq	FISH in process	unknown
6	del Xq/Yq, dup Xp/Yp	46,X, der(Y)	de novo
7	dup Xp/Yp	FISH in process MLPA positive in mother and brother	maternal

CONCLUSIONS: The high frequency of subtelomeric aberrations detected confirmed the important role played by these rearrangements in the aetiology of Mental retardation. *De novo* aberrations are likely related to the clinical presentation of the patient while inherited aberrations probably are not the cause of the patient phenotype. MLPA has demonstrated to be a very useful method that can be offered to all mentally retarded patients in order to approach to genetic diagnosis.

**P02.002****Unusual 17p subtelomeric micro-duplication**

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We report a 5 ½ year-old girl with developmental delay, microcephaly, cardiomyopathy and distinctive facial features. Additional brain MRI findings include a hypoplastic corpus callosum and enlarged cisterna magna. Standard karyotype was normal. MLPA assay with commercial probe kits to detect subtelomeric rearrangements identified a *de novo* 17p micro-duplication. Microarray analysis with a custom made chip, which contains 1905 BAC clones covering subtelomeric and pericentromeric regions of each chromosome and regions related to known syndromes, confirmed the presence of a duplication of > 6.2 Mb confined to 17p13. Interestingly, the duplicated fragment is not continuous but interrupted by a 0.45-1.1 Mb segment that is not duplicated, suggesting a more complex rearrangement, possibly mediated by a balanced rearrangement in one of the parents. Although partial 17p trisomy has been found in association with other rearrangements, to our knowledge only one other case has been reported with a 17p13 duplication exclusively and clinical features overlapping those of our patient. Further studies on this child and her parents are currently under way and the results will be presented.

**P02.003****Subtelomeric study of 109 patients with developmental delay, dysmorphology and/or congenital anomalies of unexplained etiology - case presentations**

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Background: Subtelomeric chromosome aberrations are acknowledged as one of the significant causes of mental retardation (MR). Their prevalence is about 5.2%, ranging from 0 to 16% (depending on the preselection criteria and the expertise of the examining clinician). Because of either their small size and/or similarity of involved segments, these aberrations are undetectable by conventional banding techniques. Hence, they can be screened for by other methods, most frequently by FISH with a complete set of subtelomeric probes or by multiplex ligation-dependent probe amplification (MLPA). We report the diagnostic yield in a series of 109 patients with an unexplained combination of mental retardation with dysmorphology and/or congenital anomalies.

Methods: Patients were evaluated for subtelomeric rearrangements using commercially available total subtelomeric FISH (TS). All had normal results of GTG-banded chromosomes at the 550-band level and were referred for TS based on clinical indications suggestive for

chromosomal aberration. In some cases, for identification or confirmation of detected abnormalities, specific microdeletion and telomeric probes were used.

**Results:** Twelve subtelomeric chromosomal aberrations were recognized, which corresponds to a diagnostic yield of about 10%. Identified rearrangements were: del(1p36); der(1)(1;3)(q44;p25)pat; der(1)t(1;22)(p36.1;q11.23)mat; del(2q37) in two cases; del(4p); der(6)t(6;7)(q27;q36)mat and der(7)t(6;7)(q27;q36)mat; der(9)t(9;20)(pter-,pter+)pat; del(9qter); del(12pter); del(22q13).

**Conclusions:** Since there is still limited knowledge of the phenotype of many subtelomeric aberrations, we believe that our study gives insight into the etiology of mental retardation by clinical delineation of subtelomeric aberrations.

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#### P02.004

##### Telomeric Submicroscopic Deletion of Chromosome 1

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Submicroscopic deletion 1ptel the most common telomeric deletion with an incidence of 1:5000 live births. Patients carrying this deletion tend to have similar phenotypes. We describe 3 patients with 1ptel deletion.

Case 1: Female, 3 years old, is an only child born to healthy unrelated parents. Pregnancy was normal. Clinical examination: dystrophic appearance. Microcephaly. Synophrys and dull face. General hirsutism and severe developmental delay.

Case 2: Female, 13 year old, first chills of healthy and non consanguineous parents.

Growth retardation and early puberty, pulmonary stenosis, sensorineural hearing loss and moderate mental retardation. Facies was characteristic of 1ptel deletion.

Case 3: Male, 2 month old, with congenital cardiac anomaly and facial dysmorphic features: facial hirsutism, short palpebral fissures and developmental delay.

In all the patients FISH analysis showed deletion 1p36.3

The 1qter microdeletion is often reported in the literature as a part of complex chromosome rearrangement. No particular feature is specifically unique. Next, we describe 2 patients with isolated 1qter deletion

Case 1: Female, 5 years old, is an only child born to healthy unrelated parents.

Proportioned growth retardation, congenital cardiac anomaly, pelvic horseshoe kidney, and duodenal diaphragm. Seizures. Severe developmental delay with facial dysmorphic features.

Case 2: Male, 8 year old, is an only child born to healthy unrelated parents.

Clinical features: Low birth weight. New born with hypotonia and microcephaly. Cerebellar vermis hypoplasia. Right kidney agenesis. Peripheral pulmonary stenosis. Cryptorchid testes. Seizures and severe mental retardation. Stereotypy. Dysmorphic features.

In both cases FISH studies showed deletion 1qter.

#### P02.005

##### Presentation of four new cases with chromosome 2q terminal deletion

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Terminal deletions of chromosome 2 may be found commonly among patients, with idiopathic mental retardation, referred for subtelomeric screening. Chromosome 2q terminal deletion (2q37) is characterized by mild to moderate mental retardation, behaviour manifestations on autism spectrum, facial dimorphism (prominent forehead, thin, highly arched eyebrows, depressed nasal bridge, and hypoplasia nasal alae, prominent columella and thin upper lip) and other major congenital malformations. Patients with most distal deletion present phenotypic features which mimic Albright hereditary osteodystrophy (AHO), including brachymetaphalangia in nearly half of all patients.

We report four patients including for subtelomeric screening. In all the patients a high resolution G-band Karyotype and subtelomeric screening using MLPA were performed. Result were confirmed by fluorescence in situ hybridization (FISH). The four cases presented a "de novo" terminal deletion at or within band 2q37, ranging from cytogenetically visible abnormalities (patient 1 and 2) to cryptic subtelomeric deletions (patient 3 and 4). Molecular characterization of the breakpoints were performed by STRs. The size of the deleted segments ranged from 2 Mb to 7 Mb.

The patient 4 showed of approximately 2Mb terminal deletion. He has limb abnormalities with short fourth and fifth metacarpals on the left hand and short first, third and forth metacarpals on the right hand. These findings are consistent with previous observations to narrow the brachydactyly critical region as the terminal 3 Mb region.

We observed no clear relationship between clinical features and the size of the monosomic region; this represents a significant difficulty in predicting phenotype for this deletion.

#### P02.006

##### High resolution SNP-array and MLPA analyses of patients with atypical 9q34 subtelomeric deletions

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Recently patients with deletions of the 9q34 region were proposed to have a recognizable syndrome with a combination of mental retardation, recognizable facial phenotype, hypotonia, brachy(micro)cephaly and other congenital anomalies, mostly heart defects in up to 50% of the cases. The EHMT1 gene has been proposed as the prime candidate gene to be responsible for these features. In a diagnostic survey of patients with mental retardation/multiple congenital abnormalities, HumanHap300 illumina SNP arrays and Multiplex Ligation-mediated Probe Amplification revealed two patients with *de novo* deletions in the 9q34 region which did not include the EHMT1 gene. A patient with moderate mental retardation and facial dysmorphologies that are also seen in the "9q34 subtelomeric deletion syndrome" showed hemizygosity for PNPLA7, MRPL41, WDR85, ZMYND19, ARRDC4. A second patient with mild hydrocephalus, a heart defect (VSD) and hypospadias but without mental retardation at the age of 5 years showed an interstitial deletion with a maximal size of exon 3 up to 14 of the CACNA1B gene (i.e. exon 1, 2 and 15 until 25 were not affected). Our data (1) emphasize the need for high resolution molecular cytogenetic analysis to elucidate the underlying genome rearrangements in patients with mental retardation or multiple congenital abnormalities (2) show that EHMT1 is not the major candidate gene in all patients with a deletion in the 9q34 subtelomeric region (3) allow further analysis of genotype vs phenotype in larger 9q34 deletions.

#### P02.007

##### Subtelomeric deletion of 1p36 detected by HR-CGH could not be confirmed by FISH

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Background Molecular High Resolution Comparative Genome Hybridization (HR-CGH) is applied to detect an imbalance in whole genome. This technique is especially successful in detecting small genomic abnormalities in patients with unexplained mental retardation and/or congenital malformations.

Objective To detect and identify the existence of any chromosome alteration in two unrelated girls with mental retardation and multiple congenital clinical features.

Methodology G-banding, HR-CGH and FISH.

Results Conventional GTG-banded chromosome analyses revealed a normal karyotype in both cases. Analysis by HR-CGH demonstrated a loss in 1p36 in two patients. However, FISH with a specific subtelomeric 1p probe detected 1pter deletion in only one patient.

Conclusion Our results showed that a combination of both CGH and FISH should always be used in the identification of 1p36 microdeletion, which is difficult or impossible by banding techniques alone

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**P02.008****Reconfirmation of terminal deletions and derivative chromosomes by MLPA subtelomeric screening**

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Multiplex Ligation-dependent Probe Amplification (MLPA) has come into wide use for subtelomeric screening as a new molecular cytogenetic technique. We used 2 kinds of MLPA® Kits (SALSA MLPA KIT HUMAN TELOMER, P036B and P070, MRC-Holland) for analyzing 21 subjects of known subtelomeric imbalances which have been already detected by G-banding. Of the 12 cases detected as additional materials of unknown origin at one of the chromosomal end by G-banding, 9 had loss of the subtelomere of the derivative chromosome and gain of the subtelomere of the other chromosome by MLPA, as we suspected. But, in the rest of 3 cases, MLPA showed only gain of the subtelomere of the other chromosomal end, but no loss of the subtelomere of the derivative chromosome. Of the 9 cases detected as terminal deletion by G-banding, 6 cases had only loss of the targeted subtelomere, as we suspected. But the other 3 cases had been detected not only loss of the targeted chromosomal end, but also gain of the other subtelomere by MLPA. We performed the metaphase FISH analysis to confirm the results of MLPA for the cases having discrepancies of the results between G-banding and MLPA. When a distal part of chromosomal arm looks smaller than the normal chromosome by G-banding, the abnormal chromosome is often interpreted as terminal deletion. But, it is noteworthy even shorter chromosome may be derived from familial balanced translocation. MLPA subtelomeric screening is useful for the precise diagnosis of structural chromosomal abnormality, and necessary for providing genetic counseling.

**P02.009****A terminal 7,1 Mb chromosome 18p deletion flanked by a 2,3 Mb duplication in a phenotypically normal mother and her microcephalic and mentally retarded son**

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Deletions of chromosome 18p are frequent and associated with broad phenotypic variability. We report an identical and cytogenetically visible terminal deletion in a mentally retarded microcephalic boy and his normocephalic cognitively normal mother.

The patient was a 13 year old boy with head circumference 1.5 cm below the 2.5<sup>th</sup> centile. Height was at the 10<sup>th</sup> centile and weight between the 25<sup>th</sup> and 50<sup>th</sup> centile. Early motor milestones were unremarkable. However, he has needed extra help at school because of problems with concentration, behaviour, and activities requiring fine motor skills. On examination, he had a single right palmar crease. He was microcephalic but not otherwise dysmorphic. His mother was of normal intelligence. She was 172 cm tall with a normal head circumference and no dysmorphic features.

G-banded chromosome analysis revealed a terminal 18p deletion, removing band 18p11.3. Array CGH analysis on Agilent 44K arrays indicated a 7,1 Mb terminal deletion flanked by a 2,3 Mb duplication; arr cgh 18p11.32p11.23(RP11-76H24->RP11-42J5)x1, 18p11.23p11.2(RP11-207E16->RP11-784M9)x3. Work is under way to determine if the duplication is inverted. Inversion would lead to generation of a new 18p terminus. The mother's karyotype was identical. The maternal grandmother had normal chromosomes. The maternal grandfather was deceased.

Inheritance of an 18p deletion has been reported previously in seven families. In all cases the deletion was inherited from the mother, possibly indicating that males with 18p deletions are more likely to express adverse phenotypic effects of this genomic imbalance. This is the first reported family in which the mother had a normal phenotype.

**P02.010****FISH analysis of replication timing of human subtelomeric regions**

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Recent studies have shown that DNA replication timing is correlated with transcriptional activity of genes. Synchronous replication of alleles was demonstrated for genes with a biallelic and asynchronous replication for those with a monoallelic mode of expression. Alteration of replication order has been associated with aneuploidy and genetic instability, while asynchronous replication with the formation of a deletion. No data on replication timing of subtelomeric regions (SR) are available so far. SRs are highly unstable and rearrangements have been reported in ~7% of patients with mental retardation.

The objective of this study was to determine the replication timing of human SR. The replication pattern of SR of chromosomes 5, 7, 8 and 20 was investigated using FISH method in lymphocytes of 4 healthy donors. At least 200 cells per probe per person were studied. The replication status of a locus was classified as unreplicated ("s"-single signal) and replicated ("d"- doubled signal).

The results showed that each SR had a characteristic timing of replication, and that 20q (ss: 47.8±3.1%; dd: 18±1.8%) was the first of examined SRs to replicate, whereas 5q (ss: 75.9±1.8%; dd: 4.3±0.5%) was the last ( $p<0.001$ ). The analysis revealed variable level of replication synchrony of homologous SR. The frequency of sd cells hybridized with the probes for the 5p, 7p, 8q, 20q (30.1±2.6%) was significantly higher ( $p<0.02$ ) compared to 5q, 7q, 8p, 20p (20.6±2.0%).

These results suggest that replication pattern might contribute to the non-random involvement of specific SR in the aetiology of mental retardation.

**P02.011****Human herpesvirus 6 always integrates at the telomere region**

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Human Herpesvirus 6 (HHV-6) has the unique ability amongst the human herpesviruses of integrating its genome in a persistent latent state into a chromosome of the individual infected. We used fluorescent in situ hybridization (FISH) to investigate the HHV-6 chromosomal integration sites in 7 unrelated individuals with high levels of HHV-6 viral load, which is indicative of HHV-6 chromosomal integration. A cocktail of 8 non-overlapping plasmids containing between 9Kb-16Kb inserts of the HHV-6 genome was used as a HHV-6 specific probe (Clark DA et al., 2006). FISH proved HHV-6 chromosomal integration in all 7 individuals, either in phytohemagglutinin-stimulated peripheral blood (PB) leukocytes or in Epstein-Barr virus-transformed lymphoblastoid cell lines derived from PB lymphocytes. HHV-6 was found in all 50 cells analyzed and 6 different chromosomal integration sites were identified. Two of the sites had already been reported: 17p13.3 and 22q13; while the remaining four, 9q34.3, 18p11.3, 18q23 and 19q13, had not been previously described. HHV-6 was found at one genome location per individual and always affecting only one chromosome homologue. The HHV-6 signals were always found in the vicinity of the telomeric region. For 9q34.3, the site of integration was further mapped using the BAC clone RP11-424E7, which locates at the end of 9q34.3 (genome address: 140,005,384-140,207,236). Both RP11-424E7 and HHV-6 signals co-localised in metaphase and interphase cells. HHV-6 also co-localised with a pan-telomeric probe. In summary, our study identifies four novel chromosomal integration sites and most importantly localises preferential site of HHV-6 integration at the chromosome telomere region.

**P02.012****High resolution oligonucleotide aCGH in a Jacobsen syndrome patient with an atypically small 6.5 Mb terminal 11q deletion supports FLI1 as an important mediator of thrombocytopenia**D. H. Tegay<sup>1,2</sup>, H. V. Toriello<sup>3</sup>, G. Parsons<sup>3</sup>, E. Hatchwell<sup>2</sup>;<sup>1</sup>New York College of Osteopathic Medicine, Old Westbury, NY, United States,<sup>2</sup>Stony Brook University Medical Center, Stony Brook, NY, United States,<sup>3</sup>Spectrum Health, Grand Rapids, MI, United States.

Jacobsen syndrome (JBS; OMIM#147791) is a MCA/MR syndrome clinically characterized by growth and mental retardation, thrombocytopenia, congenital heart defects, trigonencephaly and dysmorphic facial features including telecanthus, downslanting palpebral fissures, a broad nasal bridge and a "carp" shaped mouth. The vast majority of reported cases are due to ~7-15 Mb de novo terminal 11q deletions typically extending from chromosome band 11q23 to 11qter. With the advent of high-resolution molecular karyotyping by microarray comparative genomic hybridization (aCGH) smaller deletions are now frequently uncovered allowing further refinement of genotype-phenotype correlations. Here we report the case of a 5-year-old girl with clinical features of Jacobsen syndrome including growth and mental retardation, thrombocytopenia, and classical facial dysmorphism as well as unilateral optic nerve hypoplasia, multicystic kidneys, aortic valve regurgitation and tethered spinal cord. This patient was found by high-density whole-genome oligonucleotide aCGH to have a small ~6.5 Mb chromosome 11q24.3-qter deletion. This deletion starts just proximal to FLI1 (friend leukemia virus integration 1) and provides additional evidence supporting FLI1 as the important mediator of thrombocytopenia in JBS.

**P02.013****19p tel (p13.3) duplication**C. Garrido<sup>1</sup>, E. Gean<sup>2</sup>, V. Català<sup>1</sup>, L. Vila<sup>1</sup>, P. Poo<sup>2</sup>, C. Fons<sup>2</sup>, E. Cuatrecasas<sup>1</sup>, M. Pérez<sup>2</sup>, A. Serés<sup>1</sup>;<sup>1</sup>Prenatal Genetics, Barcelona, Spain, <sup>2</sup>Hospital Sant Joan de Déu, Barcelona, Spain.

Submicroscopic rearrangements involving chromosome 19 are very uncommon, there are very few reports in the literature of patients with partial trisomy of distal 19p. We describe two cases with 19p duplication.

Karyotype were normal in both patients but the availability of subtelomere specific FISH probes has made identification of cryptic subtelomeric rearrangements possible.

**Case 1:** Male, second child born to healthy unrelated parents, intrauterine growth retardation (IUGR) detected in third trimester and low weight at birth. Severe developmental delay and seizures, normal hands and feet, and very dysmorphic face.

Duplication of 19p tel using Telotyssion panel (Vysis) was observed. The extra 19p tel signal was detected at p arm of an acrocentric chromosome of group G.

Parents were studied and showed normal hybridization results. Duplication of 19p tel was "de novo" in the proband.

**Case 2:** Male, first child from non consanguineous parents, IUGR detected in the third trimester and low birth weight. Severe developmental delay, epilepsy and gastroesophageal reflux.

FISH studies showed a duplication of 19p tel and a deletion of 17q tel. The extra signal 19p tel is contained within the terminal long arm of the deleted chromosome 17q.

Parents' studies have to be done.

**P02.014****Screening for subtelomeric rearrangements in mental retardation. Description of two new cases: dup16qter and del19qter**N. Baena<sup>1</sup>, L. Comadran<sup>1</sup>, I. Crespo<sup>1</sup>, E. Gabau<sup>1</sup>, M. Roselló<sup>2</sup>, M. Guitart<sup>1</sup>;<sup>1</sup>Corporacio Sanitaria Parc Taulí, Sabadell, Spain, <sup>2</sup>Hospital Universitario La Fe, Valencia, Spain.

Copy number changes of subtelomeric chromosomal regions are responsible for 5-10% of all mentally retarded (MR) patients. Multiplex-Ligation Probe Amplification (MLPA) is a technology used to detect microdeletion and microduplication syndromes.

In the current study we determined the frequency of subtelomeric changes in a series of 108 patients showing MR and dysmorphic features in which G banded karyotype at a 600-band level were normal.

Subtelomeric assay using MLPA with SALSA P036B/C and P070 kits, and FISH in some cases were performed.

MLPA revealed subtelomeric changes in 9 out of 108 MR patients (8.3%), 7 deletions: 1p, 1q(2), 18p, 19q, 22q(2), one duplication 16q, all of them *de novo* abnormalities, and one case with a concurrent duplication and deletion 6q/3p inherited from a parental translocation. Among these imbalances we report two rare cases. 1.-Duplication of chromosome 16q in a 2-year-old girl with a psychomotor delay, who showed intrauterine growth retardation and ventriculomegaly in prenatal examination; plagiocephaly, delayed closure fontanel, hypertelorism, epicanthus, small nose and mouth, tapering fingers and hypotonia. 2.-Deletion of chromosome 19q in a 5-year-old boy with speech impairment and behaviour disorder, high palate and narrow palpebral fissures.

Comparing clinical features, only one case of dup(16)(qter) (Ahn, 2007), and one case with del(19)(qter) (EUCARUCA database) reported, both phenotypes are different from described in our patients. These two new cases could contribute to a better clinical characterization of these subtelomeric imbalances but more cases are necessary. Supported by the grant from Fundació Parc Taulí of Sabadell.

**P02.015****MLPA as screening method for the detection of cryptic subtelomeric rearrangements in patients with idiopathic mental retardation**

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Background: Submicroscopic chromosomal rearrangements involving the subtelomere regions are considered to be a significant cause of idiopathic mental retardation (MR). The Multiplex Ligation dependent Probe Amplification (MLPA) analysis has increasingly been used as an adjunct to routine cytogenetic testing and a relatively low cost and high throughput screening for the detection of small rearrangements. Objective: To screen for submicroscopic subtelomeric aberrations by MLPA method. Results: We have studied a series of 50 unselected patients with mental retardation and negative chromosomal analysis. The MLPA with SALSA P036C and SALSA P070 probe mixes was performed for subtelomere screening. Unbalanced chromosomal rearrangements detected by MLPA were confirmed by quantitative fluorescent-PCR (QF-PCR). The MLPA screening revealed chromosome aberrations in two (4%) cases: one patient with terminal deletion in the long arm of chromosome 22 and one case with double subtelomeric aberration consisting of a 12p deletion associated with 22q duplication. Conclusion: MLPA screening is a fast, sensitive and cost-effective technique for screening idiopathic mentally retarded patients with normal karyotype. Validation by another cytogenetic or molecular method is still needed for use in routine diagnostics.

**P02.016****Array-CGH analysis in 48 patients with complex syndromic phenotypes**E. F. Belligni<sup>1</sup>, J. Messa<sup>2</sup>, A. Vetro<sup>2</sup>, C. Migliaccio<sup>1</sup>, N. Chiesa<sup>1</sup>, C. Molinatto<sup>1</sup>, G. A. Delmonaco<sup>1</sup>, G. B. Ferrero<sup>1</sup>, O. Zuffardi<sup>2</sup>, M. Cirillo Silengo<sup>1</sup>;<sup>1</sup>Department of Paediatrics, Torino, Italy, <sup>2</sup>Department of Genetics - University of Pavia, Pavia, Italy.

Array comparative genomic hybridization (array-CGH) detects DNA copy number variations, allowing to identify genetic imbalances in human genetic disorders. We present the results of array-CGH analysis in 48 children affected by mental retardation, congenital malformations, and dysmorphic features. Standard karyotype was normal in all cases but 3, array-CGH analysis detected a chromosomal imbalance in 19 patients: 7 deletions, 3 duplication, 1 tetrasomy, 2 double deletion, 1 double duplication and 5 more complex chromosomal rearrangements. In a patient affected by a complex PEHO-like syndrome, characterized by severe developmental delay, severe seizures, hyporegenerative anemia, hypoalbuminemia and specific facial dysmorphisms, a *de novo* duplication of 22q11.23 has been identified, allowing to define a putative critical region for this complex developmental disorder. The analysis confirmed the clinical diagnosis of five genomic syndromes (Smith-Magenis, del1p36.33, del9q34.3, X-linked ichthyosis, Pallister-Killian), and in 3 patients it identified a complex chromosomal rearrangement previously described as a simple chromosomal anomaly by standard karyotype. Ruling out the four cases in which the chro-

mosomal rearrangement could theoretically be performed by region specific FISH, array-CGH detected 15 rearrangements in 44 patients, with a high detection rate (34,09%), allowing to propose a new critical region for a complex syndromic PEHO-like phenotype.

#### P02.017

#### Defining a new checklist of sensitive clinical signs in mental retardation, useful to select patients for array-CGH and to validate array-CGH results

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Cryptic chromosomal rearrangements are responsible of about 1-25% of cases of idiopathic mental retardation.

We studied 325 subjects with mental retardation/developmental delay by the means of subtelomeric FISH (300 patients) and array-CGH analysis (70 patients) using BAC-array with an average resolution of 1 Mb.

Cryptic chromosomal rearrangements were detected in 29 cases (10%) by subtelomeric FISH. A total of 70 patients with normal telomeres underwent array-CGH, that disclosed an interstitial cryptic abnormality in 17 (24%). Adapting this percentage to all cases with normal telomeres, detection rate for cryptic chromosome abnormality was 30% in the present cohort of patients. It represents the highest detection rate of cryptic chromosome abnormalities in idiopathic mental retardation reported so far by molecular karyotyping.

Detailed clinical analysis of all positive cases and of 50 negative patients allowed us to develop a new checklist of the clinical signs sensitive for chromosomal abnormalities. It includes 5 categories: 1) postnatal growth abnormalities, 2) disproportion between growth parameters, 3) minor facial anomalies, 4) hands and feet abnormalities and 5) major malformations. A 0-2 score was assigned to each categories, with a maximum of 10. Cut-off value resulted to be 4 in our series. Interestingly, familial MR and IUGR were not sensitive signs. Genotype-phenotype correlations represent a great problem during array-CGH analysis.

We propose this checklist as an useful tool for clinical validation of the chromosomal unbalances detected by array-CGH.

#### P02.018

#### Identification of cryptic chromosomal rearrangements in patients with multiple anomaly syndromes and mental retardation using oligo-based array-CGH

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Chromosomal abnormalities are the major cause of mental retardation (MR), growth and developmental delay and dysmorphic features. Many of these imbalances are caused by submicroscopic deletions or duplications not detected by conventional cytogenetic methods. Array-CGH is an innovative high-resolution technology that detects and maps submicroscopic DNA copy number alterations, improving the diagnostic detection rate of subtle copy number changes.

From 67 patients with mental disability, congenital anomalies, dysmorphic features and unknown underlying cause investigated by G-banding, FISH, SKY and high-resolution CGH (HR-CGH) were chosen 4 that were also screened using 60-mer oligonucleotide-based array-CGH (Agilent). Two interstitial and two terminal imbalances were identified. In patient 1 and 2, both with normal karyotype, *de novo* interstitial deletion was found at 6q15 and 11q13, respectively. Both abnormalities arose *de novo* and were previously detected using HR-CGH. Array-CGH studies not only confirmed these aberrations but specified their position and size. In patient 3 with no cytogenetic finding, array-CGH revealed deletion of terminal part of 1p include 1p36. The deletion was *de novo* and was confirmed by FISH and MLPA methods. The last patient had karyotype 46,XY,der(4p), but no evident cytogenetic imbalance. Using array-CGH, not only deletion of telomeric region of 4p, but additional duplication of terminal part of 8p was revealed. Both imbalances were also detected by MLPA, but duplication at 8p was

negative by FISH.

We conclude that oligonucleotide based array-CGH can be used as excellent diagnostic tool for genome-wide screening and identification of cryptic chromosomal imbalances not evident by routine cytogenetic analysis.

#### P02.019

#### High resolution Agilent 244K oligoarray CGH analysis for screening of patients with congenital eye malformations

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Hereditary diseases of the eyes are a frequent cause for blindness in early childhood with complex etiology. The exact frequency of chromosomal deletions and duplications causing congenital eye abnormalities is unknown. Summarising results from conventional karyotyping showed that around 1/8 segmental chromosomal duplications and 1/6 deletions are associated with eye malformations. Considering the high frequency of microscopically visible imbalances associated with eye disorders we hypothesize that patients with idiopathic eye anomalies may carry submicroscopic imbalances.

Compared with conventional cytogenetics methods - karyotyping and fluorescent in situ hybridization [FISH], array Comparative Genomic Hybridization [array CGH] provides the advantage of full genome scan with significantly higher resolution to detect deletions or duplications. Agilent 244K oligoarrays allow to perform a genomic screen with a theoretical resolution higher than 50kb.

We screened patients with congenital eye malformations and associated abnormalities and their both parents on 244K array. The project aims to identify novel genes involved in the development of the eye and to improve the diagnosis in these patients. We will present results from the analyses.

#### P02.020

#### Inherited not polymorphic CNV in mental retardation patients: implications in clinical practice

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Mental retardation is a common disorder, affecting 1-3% of the population. In spite of all diagnostic tools available, the etiology can still not be established in half of the cases. The introduction of array-CGH analysis has improved the identification of novel genomic disorders. However, this high-resolution new technique open novel diagnostic challenges when inherited private CNVs of unclear clinical significance are found. The analysis of 84 patients with mild to severe mental retardation associated to facial dysmorphisms and/or congenital anomalies revealed 10 private CNVs inherited from an healthy parent. Three were deletions (7q31, 14q21.1, Xq25) and 7 duplications (12p11.22, 12q31.31, 13q31.1, 17q12, Xp22.31, Xq28) ranging between 0.1 and 3.8 Mb. Three small rearrangements were gene desert. The remaining 7 had a mean gene content of 5 (ranging from 1 to 18). None of the rearranged genes is known to be imprinted. Three disease-genes were found in three different cases: *KAL1* in dupXp22.31, *STS* gene in another dupXp22.31 and *TCF2* gene in dup17q12. The patient carrying the last duplication presents, among others, sex reversal, Peters' anomaly and renal cysts and the duplication is located 4Mb apart of the *HSD17B1* gene, coding a key enzyme of testosterone biosynthesis. We suggest that at least in this case low penetrance instead of no pathogenesis, should be taken into account. We discuss on the final interpretation that should be given in the clinical practice and the opportunity to report such rearrangements in the family reports.

#### P02.021

#### Description of two new cases of microdeletions detected by array-CGH in carriers of apparently cytogenetically balanced chromosome rearrangements associated with phenotypic abnormalities

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Cytogenetically balanced chromosome rearrangements constitute chromosome abnormalities that may be associated with mental retardation and dysmorphic features, suggesting possible cryptic imbalances in the breakpoint. Due to the limited resolution of conventional cytogenetics, new molecular techniques are required for their detection, such as array-CGH.

We present two patients with phenotypic abnormalities and *de novo* cytogenetically balanced rearrangements. After analysing them by 1 Mb array-CGH, imbalances were observed in both cases (just one case involving the breakpoints).

In patient 1, who presented deafness, hydrocephaly and coloboma, the karyotype was 46,XX,t(6;13)(q23;q31)dn. Array-CGH detected a 13q32.2 deletion, 13q33.1 duplication, 1q44 duplication and 16q21-22 deletion. The last one is the only confirmed abnormality by FISH for the time being (BACs RP11-89O14 and RP11-46F3), but it has been described as a polymorphism.

Patient 2, with a Silver-Russell-like phenotype, showed the karyotype 46,XX,inv(7)(q21.12q34)dn. Array-CGH did not show any deletion in the breakpoints, but highlighted a *de novo* microdeletion of 5Mb at 3p12.3-3p13, confirmed by FISH (BACs RP11-781E19 and RP11-59O5).

In relation to patient 2, the only confirmed case for now, a review of similar reported cases has been performed. Although none of them has an identical microdeletion, common features have been observed, ranging from minor anomalies to growth and mental retardation, and brain, heart and lung malformations. Many genes of 3p12.3-3p13 region remain still unknown, making it difficult to establish a good genotype-phenotype correlation.

These results underline the importance of the use of molecular cytogenetics in patients with apparently cytogenetically balanced chromosome rearrangements associated with phenotypic abnormalities.

## P02.022

### Oligonucleotide array-CGH in postnatal cytogenetics

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High-resolution array-CGH (aCGH) allows the detection of small, sub-microscopic imbalances in euchromatic chromosomal segments. We have performed aCGH for the detection of constitutional imbalances in 129 patients with normal karyotype and developmental delay and/or various malformations, dysmorphisms, or complicated epilepsy of unknown etiology using 44K, 105K, and 244K oligonucleotide arrays (Agilent). In 22 of 129 patients euchromatic imbalances have been detected (~17%) by aCGH and verified by FISH analyses. In seven out of 18 cases where parental samples were available for verification analyses, it could be shown that the euchromatic imbalance (3 deletions: 0.4 Mb-1.9 Mb; 4 duplications: 1.3 Mb-5.3 Mb) has been transmitted from a phenotypically normal parent. Overall, in 14 of 129 patients (~11%) a *de novo* aberration has been detected; some of those representing well known, others recently established microdeletion and microduplication syndromes. In addition to the 129 patients, 19 patient samples were analyzed to further characterize a chromosomal aberration detected initially by conventional cytogenetics. In eleven of these cases (~58%) including complex translocations and ring chromosomes, the imbalance(s) could be characterized successfully in more detail using aCGH. Hybridization of microdissected chromosome material of a supernumerary ring chromosome 19 onto a 105 K array allowed the high resolution mapping of the nonlinear euchromatic content. However, in eight preselected cytogenetic samples no euchromatic imbalance could be detected by aCGH using whole genomic DNA. In summary, aCGH and array-hybridization of microdissected chromosomes are highly informative methods for the detection and characterization of chromosomal imbalances in postnatal cytogenetics.

## P02.023

### Array CGH detection of genomic imbalances in 370 patients with unresolved retardation syndrome

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Genomic imbalances are a major cause of mental retardation and developmental delay in patients with congenital and developmental abnormalities. With the advent of whole genome array-CGH analysis, the number especially of interstitial genomic imbalances increased dramatically. Here we present our results of array-CGH investigations in 370 cases of patients with retardation syndromes and dysmorphic features using the Cytochip v2 BAC array (BlueGnome, Cambridge). Among these cases we found 55 genomic imbalances (15%). Seven (2,1%) of the genomic imbalances were telomeric (3 deletions, 4 duplications) and 48 interstitial (12,9%, 28 deletions, 12 duplications). Of these interstitial imbalances 28 were >1 Mb in size (average 3,5) whereas 20 were smaller or equal to 1 Mb in size. Four aberrations included terminal deletion/duplication events (three *de novo*, one inherited from a balanced father). 8 cases (2,4%) were patients with known microdeletion or microduplication syndromes (two microdeletions 22q11.2, two microdeletions 17p11.2, two microdeletions 15q11.2q13, two microduplications 17p11.2). FISH has not been performed prior to the array CGH, as no obvious indication of the referrals existed. All of the detected gains and losses have been confirmed by either FISH or MLPA. A small 0,8 Mb deletion in the long arm of chromosome 2 (2q22.3) lead to the molecular genetically confirmed diagnosis of Mowat-Wilson syndrome. To summarize, array CGH is a very powerful tool to detect genomic imbalances in up to 15% of previously unresolved cases.

## P02.024

### Genome-wide analysis of DNA copy-number changes in subjects with mental retardation

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During the last three years we analyzed more than 1000 individuals affected by mental retardation and/or congenital anomalies by oligo-based array-CGH. About 300 of these subjects had a known imbalance already defined by conventional cytogenetics and the genome-wide analysis helped us to better characterize these rearrangements. A group of 703 individuals with normal karyotype was investigated by using two different microarrays (Agilent 44K and 244K) which have an average resolution of about 100Kb and 20Kb respectively. Among those investigated by the 44K chip (557), 126 (22,6%) subjects were unbalanced and 82 (14,7%) had imbalances causative of their phenotype. 146 patients were investigated through the 244K chip; 46 (31,5%) were unbalanced and 22 (15%) such imbalances were pathogenic. We are not able to establish whether the remaining 66 imbalances were causative or not. In fact most of them were inherited from a normal parent whereas in few cases we could not investigate the parents. Interestingly, our study highlighted some peculiar rearrangements such as *de novo* triplications or two independent genomic disorders in the same patient. On the other hand, our study demonstrate that increasing the resolutions may improve the detection rate, although the percentage of potentially causative imbalances detected using these two platforms is not significantly different.

## P02.025

### Validation of BAC-array CGH using a different array CGH platform

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Array CGH utilizing spotted BAC/PAC clone microarrays has recently been introduced as a valuable diagnostic tool for the detection of chromosomal imbalances in patients with mental retardation and developmental delay. Since diagnostics generally requires a binding medical opinion, results from array CGH analyses need to be validated to avoid false positive results. The currently most widely used validation method is FISH of metaphase spreads using the same BAC/PAC clone that has been identified as conspicuous on the chip array. Whereas FISH is the method of choice for the validation of deletions, duplications are generally difficult to interpret. We therefore used a second, oligonucle-

otide-based array platform (244K chip, Agilent, Santa Clara, USA) on a series of 10 cases that had prior been analyzed on the CytoChip version 2.0 (BlueGnome, Cambridge, UK) and partially validated by FISH. Our findings indicate that cross validation using a second array CGH platform is a suitable approach for the validation of BAC/PAC array results that combines the advantages of other validation methods such as FISH, qPCR or MLPA. The cross validation approach is characterized by a robust protocol, a rapid work-up, no need for metaphase spreads and moderate costs.

#### P02.026

#### Characterization of balanced chromosome translocation breakpoints associated to phenotype by microdissection and aCGH

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The positional cloning of genes affected by cytogenetic anomalies associated with phenotype is one of the main methods for the identification of disease-related genes. However, mapping translocation breakpoints and rearranged chromosomal boundaries by molecular cytogenetics is labour intensive. The isolation of the aberrant chromosomes by either flow sorting or microdissection permits the specific analysis of their genomic content by high throughput technologies such as aCGH or sequencing. We have applied chromosome microdissection and aCGH to map three apparently translocation breakpoints associated with different phenotypes: 1) 46,XY,t(4;15)(q22;q26) in a chronic myelomonocytic leukaemia; 2) 46,XX,t(11;13)(q21;q14) in a patient with language and developmental delay; and 3) 46,XY,t(7;18)(p15.1;q21.1) in a male with hypogonadotropic hypogonadism. We first isolated one of the two derivative chromosomes (6-8 chromosomes), amplified the DNA by DOP-PCR, and performed reverse FISH in order to verify the appropriate cytogenetic location of the microdissected material. Then, we hybridized the DNA onto a BAC array and the Agilent 244K CGH oligoarray for precise mapping. All three translocation breakpoints have been narrowed down to regions of ~8-15 Kb in each chromosome with high reproducible signals. We are currently attempting to further define the exact breakpoints by either tiling array or direct PCR and sequencing. In summary, the manual microdissection of aberrant chromosomes is a cheap and reliable method to obtain target DNA for further high throughput analysis with microarrays.

#### P02.027

#### High Resolution Comparative Genomic Hybridization analysis in patients with Idiopathic Mental Retardation

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Mental retardation (MR) and development delay (DD) occur in 2-3% of the general population and are very heterogeneous entities. Clinical characteristics observed in these patients are not always related to a specific syndromes, so different technologies must be applied to investigate the origin of MR. Conventional karyotyping has a resolution of >5 Mb and detects chromosomal alterations in >5% of individuals with unexplained MR, while Comparative Genomic Hybridization (CGH) is a molecular cytogenetic technique that can characterize unbalanced genetic material in a one-step global screening procedure. CGH analysis also provides information about the origin of gains and losses of chromosomal material and maps these imbalances to their position on the chromosome. We have used high resolution CGH to detect deletions and duplications in the range of 2-5 MB in order to find genomic imbalances in 10 patients with idiopathic mental retardation. The analysis by HR-CGH reveals 8 cases with normal profile and two showed abnormal results, one with a gain in 11p15.1 and the second one a loss in 12q21.3. These cases illustrate the value of molecular cytogenetic techniques as an important tool in the diagnosis and assessment of MR as well as a phenotype-genotype correlation.

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#### P02.028

#### Array-CGH diagnosis of cryptic chromosome aberrations

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Chromosome abnormalities are the most frequent cause of congenital malformations/mental retardation syndromes. Recently it was demonstrated that molecular karyotyping improves the detection rate of sub-microscopic aberrations up to 5-17%. Here we present the molecular and clinical data of three unrelated individuals with different chromosome aberrations, depicted by array-CGH analysis.

The smallest described so far deletion 1q44, only 200 kb in size, appeared *de novo* in a 4-year-old girl with clinical features inconsistent with the currently known phenotype of 1qter deletion syndrome. She presented mild mental retardation, speech delay, epilepsy, persistent foramen ovale and facial dysmorphism.

A 123 kb duplication of 8p11.1 chromosome region was detected in a severely mentally retarded 13-year-old boy with microcephaly, down-slanted palpebral fissures, midface hypoplasia, bird-like nose, long philtrum and retrognathia. Very few patients with duplications involving the same chromosome region were reported till now. Surprisingly, both the mother and healthy brother had cytogenetically balanced translocation t(9;22)(q22.1;q13.1).

Duplication of CHKAD clone (Shaikh et al., 2000) within the critical Di George region was found in an 11-month-old-boy. He was a product of twin pregnancy and had developmental delay, large fontanel, hypertelorism, depressed nasal bridge, anteverted nostrils, long philtrum, microretrognathia and inverted nipples. Few mentally retarded individuals with 22q11.2 duplication, but larger in size, approximately 3-6 Mb, were described. The phenotype was variable, characterized mainly with neurological disturbances.

The reported herein chromosome aberrations, smaller than 1 Mb, contribute to the interpretation of such anomalies in the clinical practice, an evolving issue after the routine application of array-CGH.

#### P02.029

#### De novo balanced chromosomal rearrangements in patients with mental retardation and/or multiple congenital abnormalities re-analysed by array CGH

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De novo balanced chromosome rearrangements, as detected by cytogenetic banding techniques, occur in approximately one in 2500 newborns. The risk of mental retardation (MR) and/or multiple congenital abnormalities (MCA) is twice as high as in random populations. It is plausible that in approximately half of these cases the observed phenotype is caused by deletion, disruption or otherwise inactivation of a gene(s) at the breakpoint region(s). As the resolution of standard cytogenetic banding techniques is approximately 5 Mb, the unbalanced nature of some of these translocations may have escaped detection. We analyzed 19 patients with MR/ MCA and an apparently *de novo* balanced chromosome rearrangement at minimal 550 banding level, by high resolution arrayCGH using an 244K oligo array (Agilent). Eighteen patients carried a translocation, one of these patients had a complex translocation involving three chromosomes. A further patient carried an inversion. With the 244K oligo array we detected an unbalance at the breakpoint regions for four patients; at 5 out of 39 breakpoints. In addition cryptic microdeletions and duplications not located at the translocation/inversion breakpoints were seen for several patients. The *de novo* nature of these aberrations is still under investigation. The clinical relevance of our findings will be discussed.

**P02.030****Investigation of cryptic chromosomal imbalances in patients with mental retardation and/or multiple congenital abnormalities using array-CGH**

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Chromosomal abnormalities constitute the major cause of mental retardation (MR). Conventional chromosomal analysis still remains the most important tool for identification of genomic imbalances causing MR, however subtle aberrations smaller than 5Mb are missed by routine karyotyping. Array-CGH was recently introduced to clinical practice, significantly increasing the detection rate of chromosomal abnormalities. The aim of the current study was to investigate 70 patients with various degrees of mental retardation and/or congenital abnormalities for cryptic chromosomal imbalances. All patients were clinically examined and tested by karyotyping and FISH, in order to exclude large chromosomal abnormalities and suspected microdeletion syndromes. Array-CGH was performed using BAC microarray Cytochip platform (BlueGnome) with a median resolution of 565kb. Clinically significant submicroscopic chromosomal imbalances were detected in 4 patients (5.7%). Abnormal results were confirmed by FISH. The percentage of identified abnormalities in the current study is lower than previously reported by other studies, probably due to the fact that no specific selection criteria were applied in our patients. One deletion, one duplication, one unbalanced translocation and one mosaic case with both a deletion and duplication were identified. One of the detected abnormalities was de novo, one was familial, while the remaining two are still under investigation. Two out of the four detected abnormalities would have been identified by subtelomeric screening and two would have been missed. Array-CGH is a powerful tool for the identification of novel chromosomal syndromes and identification of new cases of known syndromes that will allow more accurate prognosis and phenotype-genotype correlations.

**P02.031****Modelling human microdeletion syndromes by chromosome engineering in mice**

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Array-based diagnostics and the pooling of information in databases such as DECIPHER has accelerated discovery of novel microdeletion syndromes. Examples include the recently described 17q21.31 and 22q11.2 microdeletion syndromes (refs 1 and 2). The identification of the individual genes responsible for the phenotype is facilitated by chromosomally engineered mouse models. We are using such models to analyze the chromosomal loci for recently described microdeletion syndromes (see Table).

Our strategy is to target MICER clones (ref 4) to the proximal and distal endpoints, respectively, of each deletion. Following Cre treatment, mouse ES cells are microinjected into blastocysts to generate male chimaeras. Phenotyping of offspring is carried out by MRI scanning at embryonic day 15.5. Where an appropriate phenotype is identified, a series of nested deletions is made in order to identify a candidate gene or genes. Ultimately, suitable candidates are tested by BAC rescue of the deletion. The 6.8 Mb mouse deletion at human 17q22-23 was made by this method and has a cardiac phenotype; generation of other mouse deletions is in progress..

Syndrome	Size of deletion	Syntenic mouse chromosome	Salient phenotypic features	Ref
Del 17q21.31	500 kb	11	MR, seizures, neonatal hypotonia	1
Del 22q11.2	2.1 Mb	16	Truncus arteriosus	2
Del 15q14	5.3 Mb	2	Cleft palate, atrial septal defect	5
Del 6q24.3-25.1	2.6 Mb	10	Atrial septal defect	6
Del 17q22-23	6.8 Mb*	11	Tetralogy of Fallot	7

\*deletion in chromosomally engineered mouse

Table: Chromosomal microdeletions amenable to modelling by mouse chromosome engineering

1. *Nat Genet*. 2006; 38(9):1032-7
2. *Am J Hum Genet*. 2008; 82(1):214-21
3. *Nature*. 2001; 410(6824):97-101
4. *Nat Genet*. 2004; 36(8):867-71
5. *Am J Med Genet A*. 2007; 143(2):172-8
6. *Eur J Med Genet*. 2007; 50(4):315-21
7. *Genetics*. 2006; 173(1):297-307.

**P02.032****Scanning copy number variations (CNV) in Angelman syndrome, mental retardation and autism**

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Angelman syndrome (AS) is a neurodevelopmental disorder with a recognizable molecular cause in about 85% of cases. Copy number variations (CNV) are an important source of variation and genes located therein are likely to have altered expression patterns, therefore contributing to phenotypic changes. Twenty AS patients without the typical molecular alterations but with well-defined clinical features were analysed by aCGH using the 244K Agilent platform. Altered regions that contained rare CNVs (<2 findings in the literature) or not reported in the Database of Genomic Variants were selected for custom Multiple Ligation Probe Amplification (MLPA) assays. We assessed the CNV status using MLPA (52 regions) in the 20 AS family trios, and expanded the study to idiopathic mental retardation (n=220) and autism (n=100). CNV analysis by MLPA was also assessed in 450 control samples. The findings included: two de novo deletions (1p36, 1q44), one maternally inherited duplication (Xp11.23), altered regions in diseases cases but not present in controls (4q31.3, 6q21, 6q26, 7q22.1, Xp11.23, Xp22.2 and Xq28), and several inherited genomic variants (5q31.2, 9q33.1, 10p12.33, 16p13.3, 17p13.1 and 19q13.41) not found neither in other samples nor in controls. Our results support the view that a considerable proportion of genomic regions showing variability in copy number could be involved in neurodevelopmental disorders. The absence of genomic abnormalities in controls of inherited genomic changes detected in cases, suggests that even if inherited, they could be responsible for some of the clinical features perhaps uncovering recessive mutations in specific genes involved in the phenotypes.

**P02.033****High-density SNP array analysis reveals novel chromosomal rearrangements in human cortical malformations**

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Approximately 200 patients with cortical brain malformations (MCD), including simplified gyral pattern (SGP), lissencephaly (LIS), nodular heterotopia (PNH) and polymicrogyria (PMG), were recruited in 15 years at our academic hospital. An etiological diagnosis was made in 40% of the cases after systematic neuro-radiologic, clinical genetic, routine cytogenetic and molecular genetic tests (Archives of Neurology, in press). In the remaining group without etiological diagnosis we tested DNA of circa 100 patients on Affymetrix 250K SNP arrays and analysed the data using the CNAG and CNAT programs to detect submicroscopic chromosomal abnormalities. We could demonstrate 18 pathogenic changes, 17 deletions and 1 amplification) in about 20% of the tested patients. Of these, 15 had PMG and 4 PNH. Two patients had both PMG and PNH; 1 PNH and 2 PMG were associated with hydrocephalus. No pathogenic abnormalities were found in the SGP and LIS group. The chromosomal aberrations were confirmed by qPCR and parental testing. Some concerned known loci for MCD like 1p36, 6q27, 22q11; among the new loci found are 2q13, 4q22, Xp11, 21q22, 22q13. These are not annotated as polymorphic CNV in the known databases of variants and contain candidate genes. The size of the rear-

rangements ranged between 12 Kb and 10 Mb. With this technique, also single gene deletions were detected, which directly pointed to the causative mutation. Our data show that this novel approach detects up to 20% genomic abnormalities in a highly selected MCD patient population, mostly in the group of PMG and PNH.

#### P02.034

#### Detection of genomic copy number changes in Estonian patients with idiopathic mental retardation

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Mental retardation (MR) is a highly heterogeneous condition with a prevalence of ~1-3%. It is caused by genetic, epigenetic or environmental factors solely or in combination. Despite extensive investigations the underlying reason remains unknown in about half of the cases.

We started the first comprehensive study in Estonia in order to find out causative factors in families with idiopathic MR and help to shed light on molecular mechanisms underlying MR. To date, we collected more than 230 DNA samples from MR patients with normal karyotypes and their unaffected family members.

Infinium-2 genotyping assay with Human370CNV BeadChips (Illumina Inc) was applied as initial screening tool for detection of DNA copy number changes and copy-neutral LOH events in the samples. Acquired data were analyzed using BeadStudio v3.1 (Illumina Inc) and QuantiSNP (Colella *et al* 2007) software. Relevant results were confirmed by RT-qPCR.

After validation of the study-platform using reference DNAs, first 100 individuals from 22 families were screened. About 35 genomic rearrangements per individual were detected, most of which are reported in the Database of Genomic Variants or present recurrently in our samples.

In three families, possible disease-related imbalances were found: 1.6Mb dup(X)(p22.31), 3.9Mb del(15)(q13.1q13.2), 8.3Mb del(7)(q31.1q32.1). The detected rearrangements were not present in unaffected family members. Cases with similar phenotypes and aberration(s) in the overlapping regions are also reported in the DECIPHER database.

In addition, several other potentially clinically significant aberrations were found. Involvement of these aberrations in the etiology of MR is currently under investigation.

#### P02.035

#### Application of two different copy-number detection methodologies - array-CGH and array-MAPH - with identical amplifiable target sequences.

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Array-CGH has become the method of choice for the detection of subtle imbalances as cause of genomic disorders. Novel array-CGH modifications were introduced in order to detect smaller aberrations by varying the type and density of arrayed target sequences. At the same time, certain limitations have become obvious, emerging the need for alternative methodologies. Array-based Multiplex Amplifiable Probe Hybridization (array-MAPH) is a recent approach, where the analysis of copy-number data is performed by conventional MAPH, followed by rehybridization to microarrays containing DNA sequences, identical to MAPH probes. Thus, targeted amplification and reduction of the complexity in genomic material is achieved.

The aim of this study was to estimate whether a probe set, initially developed for array-MAPH, is potentially useful for array-CGH. The same human chromosome X specific probe set was applied to compare array-CGH and array-MAPH performance and to further evaluate the potential of array-MAPH methodology to be used for genome-wide identification of locus copy-number changes.

Normal male and female DNA samples were studied, as well as three patients with known chromosome X aberrations: two patients with a 12-Mb deletion and one patient with 1-Mb duplication. Our data has

proven that array-MAPH clones can be also efficiently implemented in array-CGH.

We suggest that efforts for upgrading genomic copy-number screening should not only focus on new CGH microarray probes, but also on introducing new platforms, such as array-MAPH, as a reliable, flexible and cost-effective alternative to array-CGH and high-density genotyping chips.

#### P02.036

#### Array comparative genomic hybridization and computational genome annotation in constitutional cytogenetics: suggesting candidate genes for novel submicroscopic chromosomal imbalance syndromes.

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Genome-wide array comparative genomic hybridization screening is uncovering pathogenic submicroscopic chromosomal imbalances in patients with developmental disorders. In those patients, imbalances appear now to be scattered across the whole genome, and most patients carry different chromosomal anomalies. Screening patients with developmental disorders can be considered a forward functional genome screen. The imbalances pinpoint the location of genes that are involved in human development. Because most imbalances encompass regions harboring multiple genes, the challenge is to (1) identify those genes responsible for the specific phenotype and (2) disentangle the role of the different genes located in an imbalanced region. We discuss our work on novel tools and databases that we recently developed to aid this gene discovery process. Identification of the functional relevance of genes will not only deepen our understanding of human development but will, in addition, aid in the data interpretation and improve genetic counseling.

#### P02.037

#### Cryptic chromosomal imbalances in patients with idiopathic mental retardation and multiple congenital anomalies

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Background: Chromosomal abnormalities are a major cause of mental retardation and multiple congenital anomalies. Array CGH studies have shown an incidence of 10-15% of formerly undetected imbalances among these patients.

Objective: To report array CGH screening of a series of 160 patients with idiopathic mental retardation and congenital abnormalities.

Material and methods: 160 patients with normal karyotype and normal subtelomeric results by MLPA were evaluated for cryptic chromosomal rearrangements by array-CGH using a high-definition microarray consisting of 44.000 probes (Agilent technologies).

Results: A total of 46 (29%) probably pathological rearrangements were detected. Of these, 11 alterations have been proved to be pathological, while the remaining are under study. Deletions and duplications are equally represented. Duplication sizes ranged from 20 to 3,859 kb (mean 1,250 kb, SD 2,276). Deletion sizes ranged from 0.4 to 10,314 kb (mean 1,369 kb, SD 2,319).

It is worth to note the occurrence of two *de novo* alterations in one patient: a 10,3 Mb deletion at 6q16 and a 360 kb duplication at 16p11. As the phenotype is comparable to other cases with 6q16 deletion, the duplication can be considered as a *de novo* polymorphism.

Conclusions: Array CGH should be considered an essential tool for the genetic analysis of patients with mental retardation and congenital anomalies. However, caution must be taken when interpreting the results in order to distinguish pathological from polymorphic imbalances.

#### P02.038

#### Identification of disease-related copy number variation (CNV) in patients with mental retardation by high-dense SNP genotyping microarrays

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We investigated 98 children referred for unexplained mental retardation using Hap550 oligonucleotides arrays (Illumina). Conventional karyotyping did not reveal any abnormality. Data analysis were performed with median normalization and genotypes-specific dosage calculation using R-scripts. We used standard deviation (mean SD: 0.15) and mean absolute deviation (mean MAD: 0.11) of the log<sub>2</sub> intensity ratios to assess data quality. We also calculated a signal-to-noise ratio (mean SNR: 5.44) in male DNA samples (median log<sub>2</sub> intensity ratio of the X-chromosomal SNPs minus median log<sub>2</sub> ratio of the autosomal SNPs divided by MAD). 254 candidate regions were evaluated with 2 or 3 quantitative PCRs each. 58% were determined to be true-positive findings. Our experiments are likely to underestimate the true positive rate because we excluded all known CNV polymorphisms. Under the assumption that all detected known CNV polymorphisms are true positive, this rate would be 89.7%. 65.5% of the false positive CNVs were detected in regions defined by <8 SNPs, 32.3% in 8-20 SNPs and 1.8% in >20 SNPs. Preliminary results revealed 15 de novo CNVs, 13 deletions and 2 duplications (15%), which varied in size from 125 kb to 13 Mb. Seven CNVs were known genomic disorders. Two CNVs overlapped approximately 0.9 Mb with a DECIPIER entry. The remaining 6 CNVs were not described before.

ID	Chromosome	Gain/ Loss	Position Start (UCSC hg18)	Position End (UCSC hg18)	Number of SNPs	Length (Mb)	Known Syndrome
1	1q44	Loss	241,559,266	241,684,687	18	0,125	no
2	17p13.1	Loss	7,054,704	7,348,051	57	0,293	no
3	13q32.2	Loss	99,000,207	99,549,209	156	0,549	yes
4	19p13.3	Loss	218,039	1,103,656	180	0,885	no
5	1q43-q44	Loss	241,477,990	24,3498,562	398	2,021	yes
6	16q22.2-q23.1	Loss	70,464,098	74,053,487	722	3,589	no
7	7q31.2-q31.31	Loss	113,212,019	117,409,113	593	4,197	yes
8	12q24.31-q24.32	Loss	120,674,768	125,508,628	875	4,834	no
9	15q11.2-q13.1	Loss	21,200,233	26,208,861	1,130	5,009	no
10	16p11.2-p12.2	Loss	21,512,681	29,223,380	1,373	7,711	yes
11	2q37.1-q37.3	Loss	233,320,827	242,656,041	2,254	9,335	yes
12	6q25.3-q27	Loss	157,812,841	170,723,055	3,545	12,910	no
13	6p22.1-p23	Loss	14,554,649	27,849,661	3,265	13,295	no
14	22q11.21-q11.23	Gain	17,345,628	22,757,878	1,155	5,412	yes
15	16q11.2-q12.2	Gain	45,096,893	53,506,358	1,453	8,409	no

## P02.039

### Elucidation of Complex Structural Variations at 17q21.3, the NSF Locus

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Multiple studies have recently described extensive structural variation in the human genome. Among the regions with most extensive variation, the 17q21.31 locus harbours a complex architecture, with several structural variants previously associated with abnormal phenotypes. This locus is the site of a ~900kb inversion polymorphism responsible for two divergent haplotypes, named H1 and H2. In addition, the H1 haplotype is divided into subtypes which show considerable structural heterogeneity. While the H2 haplotype has been associated to one microdeletion syndrome causing learning disability and dysmorphic features, certain H1 sub-haplotypes have been linked to neurodegenerative disorders, including progressive supranuclear palsy and frontotemporal dementia. By using published copy number variation data on 270 HapMap individuals, we have defined several structural variations within the 17q21.31 locus, including a duplication polymorphism involving the 5' portion of the N-ethylmaleimide sensitive factor (NSF) gene and a second distinct CNV upstream of NSF, which is detected only in European individuals on both H1 and H2 alleles. CNV breakpoints in each sub-type were also delineated using high-resolution oligo array. We have further developed a set of quantitative PCR assays to resolve the different H1 and H2 sub-haplotypes. Given the previous association of the 17q21.31 locus with disease and the presence in this interval of several genes related to the brain function, this new genotyping assay will be a valuable resource for testing the involvement of H1/H2 subtypes in neuro-psychiatric disorders.

## P02.040

### A Novel familial 2q32 duplication associated with distinct behavioural phenotype

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We have identified a patient with a novel 4 megabase duplication of 2q32.2-q32.3 chromosome region using GeneChip 250K Nsp SNP array from Affymetrix. The proband has global developmental delay, speech delay, and is aggressive with violent behaviour towards others and a tendency towards self-mutilation. She has foraging behaviour leading to obesity. She has striking blonde, dry, wiry hair, straight eyebrows and low-set ears. She has brachydactyly, and 5<sup>th</sup> finger clinodactyly. Her feet are broad, with a sandal gap, short toes and mild 2/3 toe syndactyly. The FISH studies have shown that this 4 megabase duplication has been inherited from her mother who has depression, and left school without any qualifications suggestive of a lower than average IQ. An unaffected sibling has not inherited the duplication. A recent report of a larger 2q32 deletion partially overlaps the duplication in this family. The duplicated region includes *INPP1*, previously implicated in bipolar disorder, and *COL3A1* and *COL5A2*, which are candidate genes for wrinkly skin syndrome but the proband does not share any characteristics of this syndrome except the mental retardation. Similarities with patients with the larger 2q32 deletion include a common behavioural phenotype and hair abnormalities. This suggests that copy number variation of 2q32 is a necessary, but perhaps not sufficient component for the behavioural phenotype and further analysis of the minimum region of overlap may help to identify important genes involved with this in particular. We discuss the clinical phenotype, review the literature, and discuss the difficult counselling issues involved where the link between submicroscopic DNA copy number change and phenotype is unclear.

## P02.041

### Confirmation of the new microdeletion syndrome of 16p11.2-p12.2

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We have identified a pericentromeric deletion, spanning about 8.2 Mb, in 16p11.2-p12.2, in a patient with DD and dysmorphic features, by aCGH. This deletion arose *de novo* and is flanked by segmental duplications. The proposita was the only child of healthy non-consanguineous parents, born after an uneventful pregnancy, at 40 weeks gestation, by normal delivery. She suffered from perinatal distress. Birth weight was 2,740 g, length 48 cm, and OFC 33 cm. Family history is non-contributory. Hypotonia and DD were present from early on. She started walking alone at 18/12. We first saw her at age 3 10/12 years. On examination, there were flat face; low-set, posteriorly rotated ears; high-arched palate; hypotonic face; left single palmar crease; long, thin fingers; a sacral dimple; and no speech. Height was at the 50th centile, weight at the 25th, and OFC at the 30th. DNA FraX, HRB, metabolic work-up, audiologic evaluation, brain MRI, electroencephalogram, heart/abdomen ultrasonography were normal. When last seen, aged 7 7/12 years, she had a mild-moderate MR, with a comprehension of a 4-year-old child, and was able to pronounce simple words and a few telegraphic phrases. Hypotonia was still present. Over the 3 9/12 years follow-up, we observed a slow, but constant, overall improvement. Our patient shows common clinical features to the four individuals described by Ballif et al (2007), and aCGH suggests that the deletions of all cases share the same distal breakpoint. Our observation validates the possibility that deletions in 16p11.2-p12.2 constitute a distinct syndrome.

## P02.042

### Complex genomic structure underlying an interrupted microdeletion in 16p11.2-p12.1 with breakpoints mapping to non-homologous LCRs

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The LCR-rich proximal chromosome 16p has been shown recently to be associated with the novel genomic disorders: the 7.1-8.7 Mb microdeletion 16p11.2-p12.1, and the proximally adjacent, recurrent

593 kb microdeletion and microduplication in 16p11.2 found in ~1% of patients with autism. The recurrent 16p11.2 rearrangements implicated in autism likely occur via nonallelic homologous recombination (NAHR) between directly oriented LCRs. The breakpoints of the 16p11.2-p12.1 deletions were mapped to nonhomologous sequences; however, the deletions were proposed to have arisen via NAHR. We present a 17-year-old patient with developmental delay, short stature, skeletal anomalies, and a normal G-banding karyotype and M-FISH. Using metaphase HR-CGH, we identified a deletion in 16p11.2-p12.1 and verified it by FISH with BAC clones. Whole genome array CGH with ~385,000 oligonucleotide probes (NimbleGen) defined the deletion between ~22,482,580-29,342,610 bp (6.8 Mb). Unexpectedly, we identified a second deletion ~600 kb in size, mapping ~750 kb distal to the first one. The breakpoints of both deletions map within nonhomologous sequences; breakpoint junction sequencing is in progress. We propose that the deletions arose through recently reported Fork Stalling and Template Switching (FoSTeS) replication-based mechanism (Lee et al. 2007) rather than NAHR. Phenotype-genotype correlation with the previously reported cases will be also presented.

#### P02.043

##### 3 Dimensional imaging in Wolf-Hirschhorn syndrome

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Wolf-Hirschhorn syndrome (WHS) has characteristic facial features resulting from terminal 4p deletions which may be small or large; or result from unbalanced translocations. Those with small deletions are less likely to have congenital anomalies. We used 3D imaging and dense surface modelling to investigate whether facial features correlate with the aetiology of the 4p deletions.

3D images and routine karyotype results were collected from patients attending support group meetings. Excluding adults and unusable images, dense surface models from 76 Caucasian WHS cases (mean age 7.7 years, range 1.4 - 18.9 years) were compared with 150 controls (mean age 7.9 years, range 0.2 - 20.4 years). The WHS cohort consisted of 19 large deletions (breakpoint proximal to 4p16.3); 17 small deletions (breakpoint within 4p16.3); 12 (4;8) translocations; 11 other translocations and 17 unclassified mainly because the breakpoint was recorded as 4p16. Pattern recognition algorithms supported 100% discrimination between WHS and control images. Eight additional cases were tested unseen, 4 interstitial deletions and 4 from different ethnic backgrounds; all were more similar to the mean of the WHS cases than the controls.

There was a significant time lag in facial growth in WHS that does not seem to depend on deletion size. Those with larger deletions had greater facial dysmorphology and greater facial asymmetry than those with smaller deletions. There is increasing interest in those few children with the smallest 4p deletions which define the WHS critical regions. We suggest 3D imaging is used for objective assessment of these crucial cases.

#### P02.044

##### Wolf-Hirschorn syndrome - case report

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Wolf-Hirschorn syndrome is a rare disease caused by the partial deletion of the fourth chromosome's short arm. Female to male ratio is 2:1. Because of the vast range of deleted material (1% to 50%) the effect on each child varies widely, but the facial appearance is suggestive of 4p- syndrome. No treatment exists for the underlying disorder and the management is supportive.

We present a nine months old female admitted in our clinic for systolic cardiac murmur. A comprehensive medical evaluation was made: prenatal and birth history, physical, neurologic and genetic examinations, biologic and imagistic evaluations (cardiac and abdominal ultrasonography, chest and skull films, brain MRI).

The patient is the second child of a young couple. She was delivered full term with a weight of 3400g and her development was normal.

By clinical examination we noticed craniofacial dysmorphism: right parieto-occipital plagioccephaly, downward lips, distinct „Greek warrior helmet” face with broad beaked nose, high frontal hairline, frontal bossing, hypertelorism; dysmorphic downward ears, bilateral preauricular tubercles, low occipital hair insertion. Skin dimples on elbows and left parasternal holosistolic cardiac murmur were present. Diagnostic evaluations show large axial and transverse skull diameters and normal sized cerebral ventricles. Small ventricular septal defect with left-to-right shunt was detected. Serologic tests for TORCH syndrome and immunoglobulin level were normal. Karyotype analysis noticed deletion in 4p15-pter region.

Conclusion: Although the patient has no growth problems or physical disabilities, nor mental retardation, constant cardiologic and neurologic follow-up care is required.

#### P02.045

##### The 4P-syndrome. A case report and literature review

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Wolf-Hirschhorn syndrome (WHS) is a rare developmental disorder associated with a deletion of short arm of chromosome 4. Well-known features of WHS are typical facial anomalies, midline defects, skeletal anomalies, prenatal and postnatal growth retardation, hypotonia, mental retardation, and seizures.

Here we report a new case of WHS who was referred to our clinic for cytogenetic investigation.

Case Report: The patient was a 9 month old baby boy with developmental delay, hypotonia, respiratory and heart problem, prominent eyes and forehead and delayed bone age.

Materials and Methods: Lymphocyte cultures from the patients were set up in RPMI 1640 medium supplemented with 20% FBS. Cell proliferation was stimulated with phytohemagglutinin. The cells were harvested after 72 h culture time. Hypotonic treatment of the cells was performed in 0.052 M KCL for 10 min at 37°C. The cells were fixed in methanol: glacial acetic (3: 1) overnight, and, after washing in fixative, were dropped onto clean slides. The slides were G-banded and analyzed.

Result :GTG banded karyotype revealed a deletion of the distal part of 4p (p15.31-pter). We describe some of the features of our case and will compare it with other reported cases.

#### P02.046

##### Pure de novo trisomy 4p: About 2 cases

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Trisomy 4p is a well-known entity as more than 80 cases have been published. Most of the reports of trisomy 4p were due to unbalanced translocations and resulting in associated trisomy or monosomy of other chromosomes. Cases with pure trisomy 4p are seldom.

We report on two cases of pure trisomy 4p arising de novo in a girl and a male foetus.

The girl aged 7 years presented with mental retardation, behaviour troubles, growth retardation, asymmetrical skull, microcephaly, triangular face, strabismus, bulbous nose, teeth malposition, abnormal shape of the ears and camptodactyly.

Standard karyotype performed on the patient and her parents showed a derivative chromosome 14 associated with a whole 4p trisomy: 46,X X,der(14)t(4;14)(p12;q11.1)mat.

The second case was a male foetus for whom a karyotype was performed because of an increased nuchal translucency and revealed an add(4)(qter). The father has a balanced translocation between the homologous chromosomes 4, his karyotype was 46,XY,t(4;4)(p12;qter). Thus, the foetus' karyotype was interpreted as: 46,XY,der(4)t(4;4)(p12;qter)pat.

Fetopathological examination at 19 weeks of gestation showed slight dysmorphic features, micrognathia, pointed chin, abnormal shape of

the ears, camptodactyly, and abnormal genitalia. Genetic counselling was given.

Although 4p-syndrome is a distinct entity, it can not be recognized without cytogenetic evaluation as the features are variable sometimes very mild.

#### P02.047

#### Clinical and cytogenetic characteristics in Wolf-Hirschhorn syndrome - considerations on five cases

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Wolf-Hirschhorn syndrome (WHS) is a rare chromosomal disease (1/50.000 new-borns) generated by a 4p deletion. We present five cases of WHS diagnosed in our service in last 10 years. In all cases we discovered the following features of WHS: marked growth deficiency, microcephaly, facial dysmorphism (ocular hypertelorism, down turned "fishlike" mouth, short upper lip and philtrum). We found cardiac anomalies in 4 cases, ocular abnormalities in 3 cases, cleft lip in 1 case and talipes in 1 case. Chromosomal analysis was performed in all 5 cases. At 3 patients, karyotype revealed 4p deletion specific to WHS. In last 2 cases karyotype was normal and we made FISH analysis in Clinical Genetic Institute in Dresden (Germany). In both cases was found a 4p16.3 microdeletion. In the last case, because of abnormal reproductive history of mother (2 spontaneous abortions, and 1 child death in neonate) the FISH analysis was made also in mother and indicated a t(4;20)(p16;p13) translocation. In this situation our patient presented in fact an association between a 4p partial monosomy and a small 20p partial trisomy. In the last case we estimate a recurrence risk about 10%. Our study reveals the importance of clinical examination in WHS, in all our cases clinical suspicion being confirmed. In addition, classical chromosomal analysis is not sufficient for confirmation of diagnosis. Thus, in all cases with strong evidences for WHS, but with normal karyotype are required a FISH analysis. Also, genetic counselling is difficult in some rare cases.

#### P02.048

#### Prenatal diagnosis of simultaneous Wolf-Hirschhorn and deletion 4q syndromes in a ring chromosome 4

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The phenotypes in patients with segmental aneuploidy are syndromes which often vary in their clinical manifestation depending on the size of the chromosomal region involved.

Formation of a ring chromosome 4, often involves loss of 4p and 4q telomeres and of more proximal regions on either or both chromosome arms. Deletions of the distal short arm of one chromosome 4 involving parts of 4p16 cause the Wolf-Hirschhorn (4p-) syndrome and deletions on chromosome region 4q33-4qter have also been recognised as a distinctive malformation syndrome.

To date, at least 17 cases with ring chromosome 4 have been described, but only two cases were prenatally diagnosed.

We report a case of prenatal diagnosis corresponding to the pregnancy of a 27 year old woman, which was referred for amniocentesis at 24 weeks of gestation because of intrauterine growth restriction (IUGR) showing a foetal biometry corresponding to a 18 weeks of gestation. Moreover, the fetus showed complex cardiac malformations.

Cytogenetic and molecular cytogenetic analysis of the cultured amniocytes revealed mosaic for a *de novo* ring chromosome 4: 46XY(10),45XY,-4(10),46XY,r(4)(p16,q35)(16).

The ring chromosome 4 was further characterized by fluorescence *in situ* hybridization (FISH) using WCP 4, CEP 4 , tel 4q, tel 4p and the Wolf-Hirschhorn syndrome (WHS).

The prenatal and postnatal clinical features are well correlated with those described for simultaneous Wolf-Hirschhorn and deletion 4q syndromes.

#### P02.049

#### Evidence for epigenetic regulation of CDKN2A in normal human placenta

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Placental development and trophoblast differentiation share many similarities with the process of tumorigenesis. These are rapid proliferation, invasiveness and gene expression profiles. It is possible, that this analogy might provided by epigenetic mechanisms. To test this hypothesis methylation status of four tumor suppressor genes in the normal human placentas was investigated. Promoter methylation of *p14ARF*, *CDKN2A*, *CDKN2B* and *RB1* genes was analyzed by methyl-specific and methylation-sensitive PCR in the cytotrophoblast and extraembryonic mesoderm of 32 first-trimester induced abortions. Studied tissues have different dynamics of epigenetic genome reprogramming and level of methylation. No methylated alleles of *p14ARF*, *CDKN2B* and *RB1* genes were detected in normal extraembryonic tissues. However aberrant methylation of the *CDKN2A* promoter was observed in 78% of cases in both placental tissues. Heretofore aberrant methylation of *CDKN2A* gene was frequently observed in different human cancers as well as in 20% of hydatidiform moles, 40% of chorioncarcinomas (Xue et al., 2004) and even up to 14% cases of spontaneously aborted embryos with normal karyotype (Park et al., 2008). Our data for the first time have demonstrated that hypermethylation of *CDKN2A* with high frequency can occur in normal human placenta. Tendency of decreasing in mean gestational age between embryos with (8.4 weeks, n=7) or without methylation (10.2 weeks, n=25) was found. It is possible, that several cell populations with epigenetic inactivation of the cell-cycle checkpoint genes can exist in placental tissues during some periods of its development providing evidence for a new unresolved mechanism of successful placentation.

#### P02.050

#### Screening for 22q13 deletion syndrome in 40 patients with clinical feautures suggestive of Angelman Syndrome and normal methylation test

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The 22q13 deletion syndrome has been associated with neonatal hypotonia, developmental and mental delay, absent or severe expressive language delay, autistic-like behaviour and subtle facial dysmorphisms. As some of those features are suggestive of Angelman Syndrome (AS), testing for 22q13.3 deletion in patients with AS features without the characteristic molecular 15q abnormalities has been suggested.

Forty patients with mental retardation, who had been previously referred with a clinical diagnosis of AS and a normal methylation test result, were included in the study. Patients had not been screened for UBE3A mutations (about 10%). Screened for 22q13 deletions applying the SALSA P188 MLPA kit contains 37 probes detecting sequences on chromosome 22q13. This kit including four probes in the SHANK3 gene, which is thought to be responsible for at least part of the phenotype, in particular the neurological symptoms.

A 22q13 deletion was discarded in 39/40 patients (97.5%) because no deletions were detected by MLPA analysis. Only one patient showed an abnormal result consisting in a deletion of the probe located in exon 9 of SHANK3 gene, but this result needs to be confirmed.

This study confirms the findings of Vries (2002) et al. suggesting that patients with an "Angelman phenotype" are not more likely to have a 22q13.3 deletion than other individuals with mental retardation.

#### P02.051

#### Phenotypic variability in Angelman syndrome - report of two cases

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Angelman syndrome is a complex neurodevelopmental disorder with a difficult clinical diagnosis and heterogeneous genetic basis.

We herewith report two cases of Angelman syndrome with different phenotypes.

First case, a 12 year-old girl, was admitted in our department for neurological evaluation because of severe progressive toraco-lumbar scoliosis, which occurred at the age of five. She also presented severe mental retardation, absence of speech, autistic features, gait difficulties with ataxia, hyperventilation episodes, and epileptic seizures. The second case, a 4 year-old boy, was referred to our department for psychomotor retardation. He had "happy-puppet" appearance, ataxic gait, and severe mental retardation. Both children were born from healthy non-consanguineous parents.

Clinical investigations included neurological evaluation, EEG, and MRI.

Chromosomal studies were performed on peripheral blood lymphocytes, by GTG banding. Locus specific BAC probes for critical region 15q11-13 and control probes were used for FISH analysis.

Normal karyotype, and 15q microdeletion was demonstrated in both cases.

In the second case, the phenotype was highly suggestive for Angelman syndrome, while in the first case, a differential diagnosis with Rett syndrome was considered, especially because of the severe vertebral scoliosis.

A diagnosis of Angelman syndrome should be considered in patients with Rett-like phenotype. Molecular investigation of specific genetic defect allowed, in our cases, the refinement of clinical diagnosis.

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#### P02.052

#### Supravalvular aortic stenosis and Williams Syndrome

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**Aim:** To present two cases of supravalvular aortic stenosis, selected from the group of aortic stenosis children, admitted in the IIIrd Paediatric Clinic in 2007, considered to be Williams syndrome. Williams syndrome is caused by the deletion of genetic material from the region q11.2 of chromosome 7, including more than 20 genes, several of these contributing to the characteristic features of this disorder.

**Material and method:** The patients were young children, one girl and one boy, extremely friendly, with moderate mental retardation, short stature, characteristic elfin face, joint laxity, systolic murmur and attraction to music. They performed clinical examination, ECG, echocardiography, cardiopulmonary X ray and angio CT, and after that they were referred to the genetician, ENT and ophthalmologic department.

**Results:** Supravalvular aortic stenosis was confirmed in both cases, associated with hypoplasia of aortic arch and large coarctation of the aorta in girl. She had from birth irreducible inguinal hernia, operated in newborn period, followed by cardiopulmonary arrest, resuscitated. The inguinolabial hernia reoccurred. She also still have feeding problems for semisolid food. Barium examination reveal large esophageal stenosis in the 1/3 inferior part. Both of them have prominent lips with an open mouth, defective tooth enamel and spaced teeth. The girl associated hypercalcemia.

**Conclusions:** After examinations, both patients are able to be considered Williams syndrome. The FISH test to confirm this syndrome has to be done. They are in follow up program and we intend, after confirmation of the genetic FISH test, to introduce them in Williams Syndrome Children Association.

#### P02.053

#### Clinical and molecular characterization of a cohort of 49 children affected by Beckwith-Wiedemann Syndrome and related congenital defects

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The Beckwith-Wiedemann syndrome (BWS) is a paradigmatic condition of overgrowth, characterized by macrosomia, congenital malformations, neonatal metabolic anomalies, and predisposition to embryonal malignancies, and a complex pattern of inheritance. Imprinting defects in 2 gene clusters located at 11p15.5 have been identified in about 75% of the cases. We report the clinical and molecular characterization of a cohort of 49 patients (27 M, 22 F) presenting a classic BWS (34) or a BWS-like phenotype (15). A molecular lesion has been identified in 16 out of the 39 patients analyzed (41%): KvDMR hypomethylation in 7 (17.9%), paternal uniparental disomy (UPD) in 6 (15.4%), partial deletion of the IGF-2/H19 imprinting center in 3 (7.7%), 2 maternally inherited and 1 de novo. All the patients presenting a genetic/epigenetic lesion were affected by a classic BWS phenotype. Seven children of the cohort (14.3%) developed an embryonic neoplasm, 5 Wilms' tumors and 2 hepatoblastomas; all neoplasms were detected in patients presenting hemihyperplasia, and two of them were diagnosed as affected by BWS only after the onset of the embryonic tumor. We confirm the complex genotype-epigenotype/phenotype correlation in BWS, describing three cases of the rare partial deletions of the IGF/H19 imprinting center, documenting a high incidence of cancer in our cohort, occurring even in children with an extremely mild expression of the overgrowth syndrome

#### P02.054

#### Molecular defects in patients with clinical symptoms of Angelman syndrome

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Angelman syndrome (AS) is a rare complex neurobehavioral disorder that is due to defects in parental genetic imprinting at the 15q11-q13 region resulting in the aberrant expression of genes located within this region. It was also suggested that mutations in *UBE3A* gene might be responsible for AS.

We included 206 patients with clinical symptoms of AS in our study. First, the analysis of methylation status was performed with MS-PCR method using primers specific for *SNRPN* locus. The abnormal (paternal only) methylation pattern was found in 23 patients and in several cases it was due to the deletion in 15q11-q13 region as shown by Methylation Specific Multiplex Ligation-dependent Probe Amplification technique.

Patients with the normal methylation pattern were qualified to the analysis of *UBE3A* coding sequence. Three missense (c.1291G>A - p.Gly192Ser, c.1249G>A - p.Ala178Thr, c.1643A>G - p.Asn309Ser), two frameshift (c.3228-3231del4 and c.1683-1689del7) and one splice-site (c.2616A>G) mutations were found in our group. Moreover, a 14bp deletion (c.3298-3311del14) in 3'UTR and stop codon change (c.3275A>G) were detected in 2 and 3 patients, respectively. In 32 (15.5%) patients, a small insertion in intron 6 (c.678 -48insT) always linked to small deletion in intron 7 (c.720 -66\_68delGAT) was identified. Therefore, the molecular analysis confirmed the clinical diagnosis of AS in 29 (14.1%) examined cases.

However, defects in other genes e.g. *MECP2*, *ATP10C* or *CDKL5*/ *STK9* might be responsible for AS specific phenotype. After careful examination of clinical symptoms selected patients would be qualified to further molecular analysis.

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#### P02.055

#### Hypomethylation gradient at the 11p15 Different Methylated Region 2 (DMR2) in BWS patients

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Beckwith-Wiedemann syndrome (BWS) is a genetic disease characterised by somatic overgrowth, macroglossia, abdominal wall defect and a variety of other findings including predisposition to embryonal tumours.

There are several molecular abnormalities associated with BWS. The majority of BWS patients (50-60%) have an epigenetic defect at the DMR2 of the 11p15 region. The epigenetic defect present in this region involves loss of methylation (LOM) of the maternally inherited copy of KvDMR (DMR2), a CpG island localised at the intron 10 of the maternally expressed gene KCNQ1.

In this same intron 10, this CpG island surrounds the promoter that drives a long transcript (KCNQ1OT1) with antisense transcription to KCNQ1. Normally, the maternal allele of DMR2 is methylated and KCNQ1OT1 is silenced. If there are epigenetic alterations in this region, loss of maternal methylation of DMR2 is accompanied by biallelic expression of the KCNQ1OT1 transcript, usually only paternally expressed.

We studied the methylation pattern by means of MS-MLPA technique (Methylation-Specific MLPA). With this technique is also possible to study the extension of the hypomethylation in the DMR2's CpG islands.

We studied 87 patients with BWS and among them we found that 37 presented hypomethylation at the DMR2 (42.5%). Moreover our results are consistent with similar studies which demonstrated that the hypomethylation includes the 5'end of the KCNQ1OT1 gene. Our findings suggest a clear pattern of hypomethylation at the KvDMR with a 5'-3' gradient of demethylation at this region.

#### P02.056

#### Laboratory progress in molecular diagnosis of Prader-Willi syndrome and Angelman syndrome - A new strategy by methylation-specific competitive multiplex PCR

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**Introduction:** Most of the Prader-Willi syndrome and Angelman syndrome patients are caused by the deletion of chromosomal 15q11-13 or uniparental disomy of chromosome 15. There are many diagnostic tools available like the FISH analysis, methylation-based PCR, multiplex PCR, High Resolution Melting analysis and commercialized MS-MLPA. Each of them has some disadvantages and limitations such as high costs, time-consuming and requiring DNA bisulfite-treatment. We introduce a novel, in-house designed methylation-specific competitive multiplex PCR, which takes three hours for the reaction, avoids bisulfite-treatment of DNA and provides reliable results of PWS and AS.

**Material and methods:** 68 patients with clinical suspicion of PWS, two with AS, and 20 unaffected individuals from the general population were analyzed. In the MS-competitive multiplex PCR, we amplified one SNRPN control gene, the KRT11 gene to serve as internal control, and the promoter of the SNRPN gene, which could be digested them with Hhal enzyme. By comparing the copy number of the three regions, we could differentiate the wild type, deletion type and UPD type PWS within one reaction. We also evaluated other different diagnostic technologies in clinical applications.

**Results:** In this article, we successfully made the molecular diagnosis of one AS patients and 46 PWS patients by several different techniques. In the MS-competitive multiplex PCR, the total copy numbers were accurately calculated in all of the cases.

**Conclusions:** The MS-competitive multiplex PCR strategy is a good alternative for molecular diagnosis of PWS and AS. This approach could serve as an alternative genotyping platform for epigenetics.

#### P02.057

#### From phenotype to mosaic paternal UPD11p15 in Beckwith Wiedemann Syndrome

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**Introduction:** Beckwith-Wiedemann syndrome (BWS; MIM 130650) is a model human imprinting disorder resulting from altered activity of one or more genes in the 11p15.5 imprinted gene cluster and defined by overgrowth, macroglossia, visceromegaly and tumor predisposition. Approximately 20% of BWS cases have uniparental disomy (UPD) of chromosome 11. **Material and methods:** We report 4 patients, three boys and one girl, clinically diagnosed with BWS. Genomic DNA was

extracted from peripheral blood lymphocytes and PCR methylation tests for 11p15.5 loci were performed to confirm the diagnosis. **Results:** All patients accomplished the consensus clinical diagnosis criteria and had ipsilateral hemihyperplasia. Transient neonatal hypoglycemia was recorded in two cases, three cases had cardiac involvement. The cases where confirmed by DNA methylation for loci LT1 and H19, microsatellite analysis was performed for one case and showed 11p15.5 mosaic paternal UPD. **Conclusions:** The consensus criteria enabled a correct and precocious clinical diagnosis in the first two months of life. The DNA tests confirmed the cases and showed that interchromatid somatic recombination was the causal mechanism for one case. Considering that all cases had a similar phenotype we assumed the same causal mechanism and established a correct tumor screening.

#### P02.058

#### Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) in routine diagnostics of Prader-Willi and Angelman syndromes

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Methylation Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) is a novel technique that allows for simultaneous detection of copy number variation and CpG methylation. The usage of probes specific for 15q11-q13 locus makes it useful as a diagnostic tool in the diagnosis of Prader-Willi and Angelman syndromes (PWS/AS). The aim of our study was the implementation of MS-MLPA technique as a standard diagnostic procedure in PWS/AS patients.

The Salsa MS-MLPA Prader Willi / Angelman Kit (ME028) was used according to MRC-HOLLAND suggested protocol. The MS-MLPA peak analysis, normalization and calculation of dosage ratio were performed with the SoftGenetics LLC GeneMarker ver 1.6 software. For the pilot study, we have chosen 20 cases with established PWS or AS molecular diagnosis based on PCR-based DNA methylation analysis of SNRPN locus (MS-PCR) and the STR analysis of deletion or UPD presence.

The results of the analysis of the copy number variation and the methylation status in 15q11-q13 locus with MS-MLPA confirmed the previous findings obtained for the pilot patients. We have also performed the copy number variation analysis in additional 16 AS and 24 PWS patients with abnormal methylation status detected by MS-PCR. The MS-MLPA technique allowed to exclude from the further molecular analysis patients with the deletion without the necessity parents testing. Therefore, it seems that the MS-MLPA is a useful method in the molecular diagnosis of PWS/AS syndromes.

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#### P02.059

#### New hypotheses in PWS/AS research: a multidisciplinary approach of rare diseases in Romania

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SPW and SA are two clinical, metabolic and neurological different syndromes with 1 case for 15000 new born. The molecular mechanisms identified imply large deletions, uniparental disomia (DUP), intragenic and epigenetic modifications in the processes of imprinting and only rare balanced translocations. The actual studies have extended the area of epigenetic modifications, involving chromatin dynamic structure through covalent modifications of their components. A group of researchers from medical centers of Romania together with APWR suggests studying new etiological hypotheses of PWS/AS.

Since a small number of cases with SPW/SA do not integrate with the etiologies described by now our project have a great opportunity to discover new and interesting aspects. The project aims to follow in these patients aspects connected to environment, diet, pollution and way of life influence trying to solve the weak points of the correct imprinting process.

Knowing the epigenetic aspects from SPW/SA will allow a more pre-

cise etiological diagnosis, an adequate genetic counseling, avoiding recurrence of the disease, epigenetic therapies and an optimal management of these diseases. These objectives are doubled by the interest concerning rare diseases in generally and the opened research wants to be a beginning in the multidisciplinary approach of rare diseases in Romania.

We are proposing to contact some teams of European researchers with certain results in the study of PWS/AS to cooperate, with the purpose of deciphering the mechanisms involved in these diseases.

#### P02.060

##### **Genomic and epigenetic abnormalities in Silver-Russell syndrome patients**

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Silver-Russell syndrome (SRS) is a heterogeneous disorder characterized by severe intrauterine and post-natal growth retardation, characteristic dysmorphic facial features and occasional asymmetry. The SRS incidence is ~1-30/100000 live-births with most cases being sporadic. Maternal uniparental disomy of chromosome 7 and maternal duplications or epimutations of the 11p15 region have been reported accounting for ~35-40% of cases. Several chromosomal abnormalities, affecting chromosomes 1, 15, 17 and X, have also been found in patients with SRS.

In order to define the molecular cause of the disease and to establish a diagnostic protocol, we have screened a collection of 46 patients with a clinical diagnosis of SRS with a "step by step" strategy. Cytogenetic abnormalities were found in three cases (r(15), sSMC(7), Xq26-q28dup). A methylation sensitive (MS) MLPA assay targeting five imprinted regions (11p15, 7q31, 15q12, 14q32, 20q13) detected epigenetic abnormalities in 4 cases, 2 with maternal UPD (verified by microsatellites) and 2 with H19 locus hypometilation (verified by F-COBRA). Screening for copy number variants was performed by MLPA (19 candidate loci), and aCGH in the still negative cases. De novo putatively pathogenic rearrangements were found in 5 additional cases (15q26 deletion, 15q26del+11q24dup, 7q36dup+ 10q, 12p13dup and 1 with 4q13.3 del). Our approach was able to detect the molecular basis of 12/46 (26%) SRS patients, further confirming the syndrome heterogeneity and suggesting novel candidate loci for the disorder. Although epigenetic abnormalities at 11p15 are less frequent in our series, the use of targeted MS-MLPA and aCGH appears to be indicated in the workup of these patients.

#### P02.061

##### **Mutations in SLC9A6 are present in about 4% of males with an Angelman syndrome-like phenotype**

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Recently, Gilfillan et al (ASHG, in press) reported that mutations in the SLC9A6 gene cause X-linked mental retardation (XLMR) with epilepsy and ataxia. Additionally, for many of the patients, the phenotype resembled that associated with Angelman syndrome. With this association between gene and phenotype, we wished to determine the degree of involvement of SLC9A6 mutations in patients with clinical findings suggestive of Angelman syndrome who had normal diagnostic testing. We tested 30 males submitted to rule out Angelman syndrome who had normal methylation studies and identified 1 mutation (3.3%). Additionally, 65 males with normal UBE3A sequencing were screened and 3 mutations (4.6%) were identified. Lastly, based on the phenotype, 20 families enrolled in our XLMR project were tested and 2 mutations (10%) were found. All of the mutations were truncating mutations. Taken together, our results would indicate: 1) SLC9A6 mutations may account for about 4% of males with a phenotype resembling Angelman syndrome and 2) SLC9A6 mutations may account for about 10% of males with XLMR associated with epilepsy and ataxia. Thus, SLC9A6

testing should be considered in any male with a phenotype resembling Angelman syndrome particularly if an X-linked pattern of inheritance is suspected.

#### P02.062

##### **Mutations in SLC9A6 cause microcephaly, epilepsy, and severe mental retardation with abnormal postures.**

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We have recently reported pathogenic mutations in SLC9A6 in 4 families with a characteristic phenotype of severe to profound mental retardation in association with microcephaly, epilepsy and abnormal postures. The differential diagnosis included Angelman syndrome in all cases but the pedigree structure suggested an X-linked mode of inheritance in 3 of the families.

There is considerable clinical overlap between Angelman syndrome, West syndrome and atypical Rett syndrome and thus we selected an additional 30 male patients from these categories and performed sequence analysis of SLC9A6 to identify causative mutations having excluded the known causes of disease in each group i.e. UBE3A, ARX and MECP2. We also screened >200 males from families with X-linked mental retardation.

We report a deletion within SLC9A6 in a pair of brothers with severe mental retardation where the mutation is likely to be pathogenic. We also report 2 sequence variants in families with X-linked mental retardation where the pathogenicity is less clear. These are a missense mutation which is likely to be a rare SNP and a splice site mutation (IVS12+4A>G) where the expression of SLC9A6 mRNA in lymphocytes is unaltered. We did not identify further pathogenic mutations in the samples from males with Angelman, West or atypical Rett syndrome cohorts suggesting that the etiology of these conditions remains heterogeneous and further genes are likely to cause these overlapping phenotypes.

#### P02.063

##### **A valuation of facial dysmorphism in diagnostics of 22q11.2 microdeletion syndrome in countries with lower average income**

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Most authors consider both specific and non-specific facial dysmorphisms as an important diagnostic criterion for 22q11.2 microdeletion syndrome. As a consequence, FISH analysis should be required frequently. While in most European countries this fact does not represent a significant problem, in those with lower average income such wide coverage is still unavailable.

The aim of this study was to compare a significance of specific and non-specific facial dysmorphism in diagnostics of 22q11.2 microdeletion syndrome.

We analyzed 34 patients that underwent FISH for 22q11.2 microdeletion in our hospital in the last three years. Among nine newborn infants diagnosed as positive, eight had some kind of facial dysmorphism. Seven had non-specific dysmorphism (88%), while only one had specific dysmorphism (12%). All six children older than one month and diagnosed as positive, had facial dysmorphism. Two of them had non-specific dysmorphism (33%) and four had specific facial dysmorphism (67%).

In conclusion, it is clear that facial dysmorphism becomes more and more specific with age. In the situation of seriously limited availability of FISH testing, some narrowing of indications possibly could be achieved in group of patients older than one month (or better, older than one year) by considering only a specific facial dysmorphism as a diagnostic criterion. However, individual approach to the patient and careful clinical assessment are necessary, as it is clear that non-specific facial dysmorphism also has an important role in clinical diagnosis of 22q11.2 microdeletion syndrome.

**P02.064****22q11 microduplication syndrome across four generations of a single family**

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Microduplication, as opposed to deletion, of the "DiGeorge region" on chromosome 22q11 is an increasingly-recognised finding, associated with a very wide phenotypic range. Patients vary in the degree of dysmorphism, intellectual disability and cardiac abnormalities, and many patients have now been reported who are classified as phenotypically normal.

We report a Northern Irish family in which the proband was diagnosed with a microduplication at the age of 20. She has mild learning problems and minimal facial dysmorphism, but has subtle abnormalities of the fingers. Her daughter developed severe seizures at birth, and has an atrial septal defect with patent ductus arteriosus. She has ocular hypertelorism, a small mandible, and significant developmental and growth delay; she tested positive for the duplication. The proband's mother and one of her brothers also carry the duplication. This makes it highly likely that one of her parents in turn also carried the duplication (these are reported as phenotypically normal; analysis is ongoing in this family).

Counselling as to the likely effects of this microduplication is very difficult, as the spectrum is so broad even within families. In particular, prenatal counselling presents significant challenges. As data continues to accumulate, more accurate risks and prevalences may be established, and potential modifying factors identified.

**P02.065****Report of three cases with the 22q11.2 proximal deletion**

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Hemizygosity for the human chromosome region 22q11.2 is associated with a wide range of overlapping phenotypes including DiGeorge syndrome, velocardiofacial syndrome. The acronym CATCH 22 (Cardiac anomaly, Abnormal facies, T cell deficit due to thymic hypoplasia, Cleft palate, Hypocalcaemia due to hypoparathyroidism resulting from 22q11 deletion) has been proposed to describe the broad clinical spectrum of phenotypes with 22q11.2 deletions. The frequency of this microdeletion is approximately 1:4000-1:8000 live births. Two types of deletions have been described. The most common, affects about 85% of patients and spans a ~3 Mb proximal region. The less common, affects about 7% of patients and spans a smaller, nested ~1.5 Mb distal region. Both types of microdeletion were found to occur as a result of nonallelic homologous recombination by means of low-copy repeat sequences located in the 22q11.2 region.

We describe the cytogenetic and molecular cytogenetic analysis of three patients having the most common proximal 22q11.2 microdeletion. Karyotype analysis from lymphocyte cultures performed by conventional G banding, at the level of 500 bands, revealed normal karyotype in all cases. Fluorescence *in situ* hybridization (FISH) analysis performed with the commercial dual probe LSI TUPLEI (22q11.2)/LSI ARSA (22q13) (Vysis) showed hemizygosity of 22q11.2 region in all three cases.

**P02.066****Prenatal detection of 22q11.2 deletion:case report with wide intra-familial phenotypic variation**

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INTRODUCTION: 22q11.2 deletion constitutes a contiguous gene syndrome that encompasses the clinical phenotypes formerly described as DiGeorge syndrome, velocardiofacial syndrome, conotruncal anomaly face syndrome, Opitz G/BBB syndrome and Cayler car-facial syndrome.

The diagnosis is routinely performed by FISH analysis.

CASE REPORT: We present a 33 years old healthy woman on is 13<sup>th</sup> gestational week that is sent to our office for genetic counseling because of a previous pregnancy that produced a newborn with Fallot te-

ralogy. Later on she had a healthy boy and a third pregnancy ended at the 9th gestational week in spontaneous miscarriage. The 450 bands karyotype of the girl was 46,XX.

The amniocentesis was performed at the 16<sup>th</sup> gestational week, and the karyotype was 46,XY. We extended the cytogenetic analysis and performed FISH of 4p16.3, 22q11.2 and 7q11.23 and we found that the fetus had a hemizygous deletion of the 22q11.2 region. The father was a carrier of the same deletion.

MATERIALS AND METHODS: FISH analysis was performed with the Vysis probe DiGeorge/UCFS (gene TUPLE1) y ARSA (control). The karyotype analysis was performed by conventional GTG banding.

DISCUSSION: This case illustrates de advantage of widening the prenatal diagnostic spectrum beyond the most common aneuploidies, tailoring the genetics tests to the family history as well as to the prenatal data. Assuming that both the Fallot tetralogy and the miscarriage were related to the familial deletion, the intrafamilial variability spans from lethal to almost normal, adding further data to the few catch 22 families studied to date.

**P02.067****Frequent 22q11 aberrations in patients with non-syndromic autism spectrum disorders shown by SNP array based segmental aneuploidy screening**

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Autism spectrum disorders (ASD) are neurodevelopmental conditions characterized by impaired reciprocal social interaction, communicative deficits, and restricted behavioral patterns. ASD occurs in syndromic forms and as non-syndromic cases frequently involving cytogenetic abnormalities. Recently, array-based genome-wide screens have demonstrated frequent copy number variation in non-syndromic ASD. Screening 56 patients with autism and additional major or minor anomalies with the Infinium HumanHap300 SNP platform (Illumina, Inc., San Diego, CA) we found in 16 patients 9 regions with deleted and 9 with duplicated signals. Aberrant signals were distributed among 16 distinct chromosomal loci. Apart from 14 patients with unique aberrations, 2 patients carried duplications and a 3<sup>rd</sup> patient a deletion within the 22q11 region, of 0.726, 2.966, and 0.388 Mb, respectively. The duplications were confirmed by multiplex ligation-mediated probe amplification and are likely to involve LCRs A, B and D. We conclude that SNP array-based screening of ASD patients uncovers an appreciable number of CNVs, which in part overlap with loci already discovered by other approaches. Our finding that 3 out of 56 ASD patients carried aberrations within the 22q11 region is highly unexpected. The relatively small size of CNVs found in this study may allow us to pinpoint candidate genes for ASD.

**P02.068****A rare recognizable 10p15 microdeletion syndrome of autism and HDR**

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The HDR syndrome is characterized by hypoparathyroidism, sensorineural deafness, and renal dysplasia. It is caused by haplo-insufficiency of the GATA 3 gene located at 10p15 and inherited as an autosomal dominant trait.

In our study we describe a patient with HDR phenotype and associated severe autism. Molecular analysis by MLPA showed a de novo heterozygous deletion at 10p15, encompassing the GATA3 gene without involvement of the DGCR2. These results were confirmed by microsatellite analysis, showing that the deleted region is located between the D10S189 and D10S1649 markers. The size of the deletion can be estimated around 2,5 Mb. The GATA3 gene has been reported as a gene important in the embryonic development of the parathyroid, renal and auditory systems. However mental retardation or autism can not be ascribed to GATA3 mutations. Therefore we can suspect that this

deleted region contains a gene responsible for the cognitive impairment observed in our patient. 10p15 microdeletions has already been described in few patients will almost identical symptoms and similar dysmorphic features. This confirms the existence of a recognizable albeit rare microdeletion syndrome with HDR and autism.

#### P02.069

#### The developing facial phenotype of the 17q21.31 microdeletion syndrome.

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The 17q21.31 microdeletion syndrome is a new genomic disorder, characterized by a 500-650 kb submicroscopic deletion.

Patients have a recognizable phenotype including neonatal hypotonia, mental retardation and characteristic facial features, most notably a long face, large prominent ears and a tubular pear-shaped nose. We present a patient with a de novo 17q21.31 microdeletion, identified by multiplex ligation-dependent probe amplification with probes hybridizing to unique sequences in 2 genes located within this region, MAPT and CRHR1.

This boy was born at term with normal growth parameters. There was marked neonatal hypotonia with feeding difficulties and necessity of nasogastric tube feeding.

Early motor milestones were significantly delayed. IQ testing at the age of 6 revealed mild mental retardation. Dysmorphic facial features noted in infancy were epicanthic folds, upslanting palpebral fissures, mild ptosis, low-set ears, a broad nose and prominent philtrum.

As the boy got older, the chin got bigger, there was marked elongation of the face and the nose became more pronounced. Subsequent pictures of our patient from age 6 months till 13,5 years illustrate this. At diagnosis age 13,5 we note a very long narrow face, large ears and a very prominent tubular pear-shaped nose with broad nasal tip. The palate is very high and there is dental crowding. We note thoracic scoliosis, wide-spaced nipples, a right pes cavus, numerous skin moles and a rather severe ichthyosis on hands and feet. He has nasal speech and a very friendly nature.

This is the first extensive description of the syndrome's developing phenotype.

#### P02.070

#### Cognitive and behavioral patterns associated with 18p-

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**Background.** While deletions of 18p- are associated with short stature and minor dysmorphic features, no clear cut cognitive or behavioral patterns have emerged. Included in our longitudinal study of persons with chromosome 18 differences is a large cohort of persons with chromosome 18p-. Adaptive behavior assessment measures everyday activities while cognitive measures assess internal cognitive processes. Currently no information is available regarding the correspondence between adaptive behavior and cognitive development for this population. **Methods.** The adaptive behavior of 10 individuals with 18p- were evaluated using the Vineland Adaptive Behavior Scales (Vineland; Sparrow, Balla & Cicchetti, 1984) and estimates of intellectual functioning were gathered using either the Differential Abilities Scales (DAS, Elliott, 1990) or the Bayley Scales of Infant Development-Second Edition (Bailey-II, Bailey, 1993). All scores were converted to standard scores with a mean of 100 and standard deviation of 15. **Results.** Intellectual functioning varied from significant cognitive impairment (N=3) to low average/average intellectual functioning (N= 3). Adaptive behavior skill development was commensurate with estimates of intellectual functioning in only three of the nine children's profiles. In the remaining six cases, estimates of cognitive development were lower than parental ratings of adaptive behavior. **Conclusions.** This study supports previous findings of significant cognitive variability within the 18p- population. It provides evidence of significant adaptive skill variability and also highlights within subject variability in cognitive and behavioral development. These findings suggest that for persons with 18p- deletions, it is critical to utilize both cognitive and behavioral mea-

sures to more fully understand development.

#### P02.071

#### Endocrine abnormalities in 18p-

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**Background.** 18p- syndrome has primarily been described in case reports and small series. An endocrine abnormality is present in ~20% of reported cases. The aim of this study was to determine the incidence and types of endocrine abnormalities in our population. **Methods.** Eleven individuals with 18p- underwent an endocrine evaluation at the Chromosome 18 Clinical Research Center (the "Center"). This evaluation included anthropometrics, bone age studies and growth (growth factors and stimulation testing) and thyroid hormone testing. In addition, a review of the available medical records for 20 additional patients was completed. **Results.** Five males and 6 females were evaluated at the Center. The average age was  $9\frac{8}{12}$  years (range  $2\frac{8}{12}$  to  $18\frac{2}{12}$ ). Six individuals were  $<5^{\text{th}}$  centile for height. Three of these were diagnosed with GHD (27% of total individuals evaluated). Ten of the 11 had normal IGF1 and IGFBP3 levels and essentially normal thyroid results (one had a slightly low T4 level, but had normal TSH levels). Thyroid and IGF1/IGFBP3 results were not available on the 11<sup>th</sup> patient. Medical record review of the additional 20 patients revealed that 8 had an endocrine abnormality, including panhypopituitarism (2); precocious puberty (1); autoimmune thyroiditis (1); treatment with growth hormone (4); and hypothyroidism (1). **Conclusions.** Taken together, 11 of the 31 (35%) individuals with 18p- included in this study had some endocrine abnormality. Thus, endocrine abnormalities are relatively common in individuals with 18p- and physicians should have a low threshold for considering an endocrinology evaluation, especially GHD.

#### P02.072

#### Cytogenetic and molecular studies of the 18q deletion syndrome in two generations with phenotypic variability

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The 18q- syndrome is one of the most common deletion syndromes, with an estimated frequency of 1/40,000 births. This syndrome is most often caused by a terminal deletion of the long arm of chromosome 18. However, individuals with interstitial deletions have also been identified. The phenotype of the disorder varies greatly between individuals, but generally includes short stature, microcephaly, midface hypoplasia, hypertelorism, congenital aural atresia (CAA), foot deformities, mental retardation (MR), and hypotonia. Some familial cases with directed transmission of the deleted chromosome have been described. Here, we describe the cytogenetic and molecular studies of the 18q deletion syndrome in a mother and her daughter. The mother presented microcephaly, developmental delay, hypertelorism, strabismus, high palate, submucous cleft palate, bifid uvula, congenital heart disease and facial dysmorphisms. The daughter presented developmental delay, cleft palate, short neck and facial dysmorphisms. Using FISH and molecular analysis with polymorphic short tandem repeats (STR), the cytogenetic karyotype could be refined showing that: mother and daughter have the same terminal deletion, the size of the deletion was narrowed and the breakpoints were mapped between the markers D18S1313 (18q22.1) and D18S61 (18q22.2), the parental origin of the deletion in the mother is paternal. This case supports that the variability in phenotype is not only determined by the size of deletion, once mother and daughter, with similar sized deletion, present different phenotypes, suggesting that allelic variation on the haploid portion of 18q, genes on other chromosomal regions or environmental factors are modulating the phenotype.

#### P02.073

#### Clinical and molecular characterization of deletions, duplications, and mutations in the 22q11.2 region

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The phenotype of patients with del22q11.2 is very variable and ranges from the more severe and complex forms of the DiGeorge and Velo-cardiofacial syndromes, to isolated heart defects or isolated mental disorders. *TBX1* and *CRKL* are genes localized in 22q11.2 which have been proposed to cause the associated phenotype. We present here the results of the characterization of deletions, duplications and mutations in the *TBX1* and *CRKL* genes in prenatal samples, newborn and children with compatible malformations or phenotype. Duplications and deletions were studied by the use of a combination of a home-brew method based on microsatellites, MLPA (SALSA 23 and 250), CGH-arrays and FISH. Mutations in *TBX1* and *CRKL* were analysed by sequencing. In total, we have identified 21 patients with a 22q11.2 deletion (1.5 and 3 Mb) and one patient with a previously not described 1 Mb duplication. In patients with no deletions or duplications we found two mutations in *TBX1*. The atypical duplication was found in a boy with a ventricular septal defect (VSD) as the sole phenotypic trait. Neither his normal parents nor his sister which is also affected by a VSD are carriers of such duplication. The size of the duplication is approximately 1 Mb and is flanked by LCR22s 3a (B) and 4 (D) a region that includes the *CRKL* gene but not *TBX1*.

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#### P02.074

##### Clinical and molecular characterization of 7 patients with 22q13.3 deletion syndrome

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In recent years the increasing use of telomere screening has provided evidences of the presence of subtle abnormalities involving telomeres in around 5% of patients with idiopathic mental retardation. This fact has allowed the recognition of new distinct clinical entities such as monosomy 1p, 1q, 2q, 9q, 14q, and 22q. In surveys of subtelomeric screening, deletion of 22q13.3 is the second most common subtelomeric deletion, after deletion 1p36.3. The prevalence of 22q13.3 deletion is not yet established. Up to the present moment more than 100 cases have been reported, and a common phenotype has emerged including global developmental delay, hypotonia, absent to severely delayed speech, autistic-like behavior, normal to accelerated growth, and dysmorphic facial features. In spite of the increasing number of cases reported, this syndrome remains underdiagnosed due to the difficulty to detect the deletion of chromosome 22 in routine cytogenetic analysis even at the 550-band level of resolution, and to the lack of a distinct phenotype and the clinical variability observed.

Here we describe the clinical and molecular findings in 7 patients with monosomy 22q13. Three of them were detected by subtelomeric screening, other two were fortuitously discovered when FISH was used to rule out DGS/VCFS, and two were observed in the karyotype and subsequently confirmed by FISH.

All of them were further studied in detail by MLPA, using the specific probe mix P188 for 22q13 deletion syndrome, and arrayCGH at approximately 1 Mb resolution.

#### P02.075

##### Phenotype of a patient with pure partial trisomy 2p(p21-->pter)

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We present the case of a 7-year-old boy with additional material on 4q. The father's karyotype confirmed that the additional segment on 4q was of chromosome 2p origin, resulting in trisomy 2p21 -->2pter. The karyotype of the proband was found as 46, XY,der(4)t(2;4)(p21;q33) pat.

His father's karyotype was 46, XY, t(2;4)(p21;q33). The mother had normal karyotype. The child was found to have dysmorphic facial features, prominent ears, long fingers, short stature, thenar and hypothenar atrophy, crowded teeth, high arched palate, prognathism, low nasal bridge, large testes, developmental delay, speech delay and seizures. This case will be compared with other reported cases of partial trisomy/duplication of 2p.

#### P02.076

##### 47,XYY/48,XXYY karyotype associated with multiple skeletal abnormalities and congenital heart disease

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We report on a 12-year-old boy with congenital scoliosis due to segmented hemivertebra between tenth and twelfth thoracic vertebrae. He had also congenital heart disease (atrial septal defect) and presented bilateral synostosis of the proximal radius and ulna associated with dislocation of both radius heads. Physical examination revealed facial dysmorphism, mild microcephaly, gynecomastia with morbid obesity, limitation of supination at the elbows, and moderate mental retardation.

Cytogenetic studies and FISH analysis showed that he had a de novo 47,XYY/48,XXYY karyotype. This case and evidence collected from the literature suggest that congenital skeletal abnormalities and heart defect may occur in the 47,XYY/48,XXYY syndrome more frequently than currently appreciated. The 47,XYY/48,XXYY phenotype may result from compounding affects of the additional X and Y chromosomes.

#### P02.077

##### Severe neurological phenotype in a boy with 48, XXYY karyotype

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A 18 month old boy was seen in our clinical genetics unit for further evaluation after having been diagnosed with 48, XXYY aneuploidy. He had a history of epileptic seizures, mental retardation, left cryptorchidism and a first degree atrio-ventricular block.

A thorough examination of the boy revealed many of the clinical features characteristically found in patients with gonosomal polysomies, as for example the truncular obesity, slight hypogenitalism, mental retardation and facial dysmorphisms (coarse face, mild frontal bossing, brachycephaly, bilateral epicantic folds and maxillary hypoplasia.). However he also presented generalized hypotonia, epileptic seizures and paroxysmal sharp-waves and polyspikes in the central occipital left hemispherical region on EEG analysis; neurological findings which have rarely described in patients with 48, XXYY karyotype.

The incidence of the 48,XXYY aneuploidy is about 1 in 50,000 newborns and initially it had been classified as a variant of Klinefelter Syndrome. Today it is defined as an independent genetic and clinical entity.

As far as we know, this is the first case report of a boy with the 48, XXYY chromosomal anomaly presenting epileptic seizures as one of the major clinical features.

**P02.078****Mental Retardation, Epilepsy and Venous Thromboembolic Disease in a Patient with a 48,XXYY Karyotype**

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48,XXYY syndrome was initially considered a variant of Klinefelter syndrome. Nowadays, it is accepted as a distinct clinical and genetic entity.

We report the case of a 27-year-old man diagnosed with cryptogenic partial epilepsy and action tremor in both hands unresponsive to beta-blockers. Due to speech and language delays and mild mental retardation a karyotype analysis was performed, which indicated that all cells were 48,XXYY.

The patient was born without neonatal complications to a healthy couple with an unremarkable family history. On beginning school, language delay and learning disabilities were detected. Language therapy helped him to develop complex language skills. He was diagnosed with asthma when he was 5 years old. At 19, idiopathic deep vein thrombosis developed in his left leg which required 8 months of oral anticoagulant therapy.

Phenotypically tall with eunuchoid habitus, gynecomastia. Craniofacial dimorphism described as a "pugilistic" facial appearance. Hypergonadotropic hypogonadism, small testes and normal penis. Leg ulcers and varicosities.

Behavioural features consist of immaturity, passivity and temper outbursts but a friendly and cooperative nature. High overall scores on adaptive scales in daily living, socialization and communication. His parents have exposed their son to a variety of activities and interests and provided consistent intervention and support when needed.

The addition of more than one extra X and Y chromosome to a normal male karyotype rarely occurs and it has its own distinctive physical and behavioural profile. There are significant variations within each polysomy X and Y group and hence no generalised prognosis can be made.

**P02.079****A 7-year-old boy with profound mental retardation, peculiar face, tracheo-esophageal fistula and bilateral sensorineural hearing loss caused by interstitial deletion 17q22-q23.3**

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Microdeletions of the long arm of chromosome 17 are rare. By our knowledge, only few cases have been reported involving deletions of chromosome 17 in region q22-q24. Common findings are microcephaly, abnormal face, anomalies of the hands, growth retardation and severe developmental delay.

Here we present a 7-year-old boy, who was born normally at term with normal birth weight 3480g and length 50cm, and microcephaly (-2 SD). Soon after the birth tracheo-esophageal fistula was diagnosed and therefore operated. At the age 7 years clinical evaluation revealed failure to thrive (-2.5 SD), microcephaly (-5 SD), profound mental retardation, stereotypic movements, peculiar facies - high forehead, palpebral fissures slant up, blepharophimosis, ptosis, hypertelorism, epicanthal folds, strabismus, broad nasal tip, high palate, low set and dysplastic ears, and preauricular pits. Additionally he had bilateral severe sensorineural hearing loss, kyphosis, scoliosis, spina bifida occulta, contractures of joints, tall fingers, partial syndactyly of II-V fingers and II-V toes, sandle gap of toes, micropenis, cryptorchid testes, and mild hirsutism. During pediatric evaluations psoriasis vulgaris, asthma, celiac disease, iron deficient anemia and mild hypothyreosis was diagnosed. We have applied Infinium-2 genotyping assay with Human370CNV-Duo BeadChips (Illumina Inc.), which showed a 5.9 Mb deletion of chromosome region 17q22-q23.3 with a breakpoint between 48,287,327 and 54,245,662bp. The aberration was confirmed by real-time PCR analysis.

Regarding to our patients findings, we suggest that a locus for sensorineural hearing loss and a locus for tracheo-esophageal fistula may be located in the region of 17q22-q23.3. This work was supported by GARLA 6808.

**P02.080****An unusual case of ring chromosome 14 with Epilepsy and developmental delay**

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<sup>3</sup>Dipartimento di Scienze Pediatriche e Medicina Clinica, University, Cagliari, Italy, <sup>4</sup>Dipartimento di Neuroscienze, Neuropsichiatria Infantile, Cagliari, Italy. We present a 10-year-old boy, born to young , healthy and non-consanguineous parents, with an unusual ring chromosome 14 associated to epilepsy and mental retardation. At the age of five months he had seizures confirmed by EEG as epilepsy. Seizures were resistant to common antiepileptic drugs. He also had microcephaly, hypertelorism , microretrognathia , large auricula , micropenis , hypotonia. Cytogenetic investigation and FISH studies of the proband revealed an unusual chromosomal mosaicism 46,XY,r(14)(p13q32)/46,XY,dup r(14),ish (14wcp+). Over 50 examined metaphases, 48 had a ring chromosome 14 and only 2 had a duplicated ring chromosome 14(4%). The abnormality r(14) is a rare cytogenetic disorder with characteristic features and episode of uncontrolled seizures. To the best of our knowledge, this is the first report describing a ring chromosome 14 mosaicism associated to a duplicated ring chromosome 14. Array-CGH analysis will be performed to detect cryptic submicroscopic rearrangements in the ring formation. We carried out this study to search for the cause of the seizures in our patient. Several hypotheses could be explain these, such as mitotic instability of ring chromosome or telomere position effect in ring chromosomes. We could also hypothesize that 14p telomere silences nearby genes on the q arm and close dependent genes involved in the seizures.

**P02.081****Partial trisomy 15q11-13 as a cause of acute onset intractable epilepsy**

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Various rearrangements involve the proximal long arm of chromosome 15, including deletions, duplications, translocations, inversions and supernumerary marker chromosome with the inverted duplication of proximal chromosome 15.

The large marker 15, that contains the Prader-Willi syndrome (PWS)/Angelman syndrome (AS) chromosome region, is usually associated with an abnormal phenotype of moderate to severe mental retardation, seizures, poor motor coordination, early-onset central hypotonia, autism and autistic-like behavior and mild dysmorphic features. Positive genetic linkage with 15q region has also been found in patients with schizophrenia. Most of the reported cases are of maternal origin.

We report here an eight year-old girl with normal intelligence who developed severe intractable seizures at age seven years. Family history was significant for a mother with episodes of acute psychosis.

The patient's leukocyte karyotype revealed 47XX+m. Her mother's karyotype looked identical.

Array comparative Genomic Hybridization (A-CGH) identified a gain of 13 BAC clones from 15q11.2 through 15q13.1, which was then confirmed by Fluorescent In-Situ Hybridization (FISH). This duplicated region is approximately 5.6 Mb in size and contains the SNRPN/UBE3A loci.

Although severe epilepsy has been described in association with rearrangements of the proximal long arm of chromosome 15, all reported cases had mental retardation.

This is the first report of a patient with intractable epilepsy and nearly normal intelligence, associated with partial trisomy of 15q11-13. She inherited this marker chromosome from her mother, who has psychiatric illness.

We suggest that this region should be tested by FISH in cases with intractable epilepsy, with or without developmental delay/MR.

**P02.082****Phenotype and 244k array-CGH characterization of chromosome 13q deletions: An Update of the Phenotypic Map**

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Genotype-phenotype correlation studies of partial deletions of the long arm of chromosome 13 have been reported previously, each focusing on few particular major anomalies, such as brain, eyes, inner organs, hands and feet, genitourinary or anorectal defects. Chromosome regions critical for some of the major malformations, however, vary noticeably and await precise delineation. Besides, data about the dysmorphic profile are limited. In order to update the phenotypic map for chromosome 13q deletions, we applied high resolution 60-mer oligonucleotide-based microarray containing 244.000 probes with a median spacing of 7.4 kb to 16.5 kb (Agilent Technologies, USA) to determine the exact breakpoints of such deletions in 14 patients. We linked the genotype to the patient's phenotype and were able to refine the smallest deletion region linked to microcephaly (13q33.3-q34), Dandy-Walker malformation (DWM) (13q32.2-q33.1), corpus callosum agenesis (CCA) (13q32.3-q33.1), the associated occurrence of both DWM/CCA (13q32.2-q33.1), meningocele/encephalocele (13q31.3-qter), ano-/microphthalmia (13q31.3-13qter), cleft lip/palate (13q31.3-13q33.1), lung hypoplasia (13q31.3 - 13q33.1), anal atresia/hypospadias/penoscrotal inversion (13q33.1-13q34), thumb a-/hypoplasia (13q31.3-q33.1 and 13q33.3-34). 'Typical' 13q-dysmorphic facial features were assigned along the chromosome, thereby mapping, the prominent nasal columella between 13q31.3 and 13q33.3. Our analyses support the hypothesis that haploinsufficiency of more than one locus within the 13q21.1-qter region is responsible for brain anomalies and neural tube defects. In contrast to previous reports of carriers of 13q32 band deletion as the most seriously affected patients, we present such individuals with a long-term survival.

**P02.083****Clinical features of a case with trisomy 10q and monosomy 3p resulting from a maternal balance translocation: A case report and review of clinical features**

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Here we describe clinical and cytogenetic data on 2 year female child with partial trisomy for the distal part of the long arm of chromosome 10 (10q22-->qter) and a concomitant monosomy 3(p25-->ppter) as a result of a maternal balanced reciprocal translocation. Her karyotype was ascertained as 46,XX,der(3)t(3;10)(p25;q22). The father had normal karyotype. The mother had an apparently balanced translocation involving chromosome 3 and 10 [46,XX,t(3;10)(p25;q22)]. To our best knowledge, this is the second reported case of partial trisomy 10q and partial trisomy 3p and first reported case from Iran. Clinical features of this case and a few published cases will be reviewed briefly.

**P02.084****Trisomy 13 mosaicism in a nine-year-old girl with mild global developmental delay**

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Trisomy 13 is associated with mental retardation and a variety of congenital abnormalities. Survival past infancy is rare because of the life-threatening medical issues associated with this condition. The phenotype of mosaic trisomy 13 is extremely variable. I report on a nine-

year-old girl who was found to have trisomy 13 mosaicism at the age of eight in the course of investigations for mild global developmental delay. She was born after an unremarkable pregnancy and delivery. Her parents first became concerned when she was two-years-old because her fine motor and speech milestones were delayed. Currently, she is integrated into a regular grade three class where she receives some remedial help; a recent developmental assessment revealed that she is functioning at the level of a six-year-old. She also has some gross motor difficulties and decreased coordination. Her general health has been good. Examination revealed that her weight and height were >97<sup>th</sup> percentile and that her head circumference was at the 80<sup>th</sup> percentile. Her facial features were slightly coarse. There was no other evidence on examination of any physical features associated with trisomy 13. The patient's karyotype was done on a blood sample and revealed that 10 out of 20 cells analyzed were trisomic for chromosome 13 (ie. 50%). This case supports the previously suggested idea that the percentage of trisomic cells, which is thought to decrease with age, correlates poorly with intellectual functioning.

**P02.085****Detection of Y chromosome and trisomy 8 mosaicism in a twelve year old girl with Turner syndrome**

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Most Turner syndrome (TS) patients show no evidence of Y chromosome sequences. According to different authors some TS patients may have Y chromosome material present in a few cells that are not detected by standard cytogenetic analysis. The rationale of identification of this low level mosaicism is of clinical importance due to the patient's increased risk of developing gonadoblastoma.

Here we report conventional and molecular cytogenetic investigations in a case of mosaic sex chromosome aneuploidy combined with mosaic trisomy 8 and the presence of low level Y mosaicism. A 12 year-old girl was presented with short stature, gonadal dysgenesis and minor craniofacial dysmorphic features.

Standard chromosome analysis on peripheral blood lymphocytes showed the presence of three cell lines, whose karyotypes were 45,X [93%], 47,XY,+8 [5%] and 46,XX [2%]. FISH analysis was performed using as probes an alpha satellite for X chromosome (CEPX, Xp11.1-q11.1), a satellite III for Y chromosome (CEPY, Yq12) and an alpha satellite for chromosome 8 (CEP 8, 8p11.1-q11.1). The results of FISH analysis were in concordance with those of conventional cytogenetic analysis showing a slight increase in the percentage of interphase nuclei revealing trisomy 8 and Y chromosome mosaicism (8% FISH versus 5% standard cytogenetics).

The importance of systematic search for hidden Y chromosome mosaicism in patients with TS is justified by the possibility of developing gonadoblastoma and it is necessary for the appropriate clinical management of the patient.

**P02.086****Isolated bilateral Ulnar Agenesis due to 4q34.1 deletion in a young male patient**

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Ulna a- or hypo-plasia is a rare condition, mostly occurring associated with other malformations as in Schinzel-Ulnar-Mammary, Weyers, or Femur-Fibula-Ulna syndromes. If isolated the disease is very uncommon and, to our knowledge, remains unexplained. We describe a 3 years old male patient with bilateral isolated ulna aplasia and 4q34.1 deletion diagnosed by Array-CGH.

This patient was the first child of young, healthy and non consanguineous parents. He had a healthy young sister. The pregnancy was uneventful. He was born at term. He had aplasia of both ulnas with bilateral but asymmetrical absence of post-axial rays. Only the thumb was

present on the right side, but the first two pre-axial rays were present on the left. Both elbows were abnormal with unusual upper extremities of the radius, and lower extremities of the humerus. No other malformation was observed even in the lower limbs. Karyotype was normal: 46,XY. At 3 years of age the patient has normal growth and psychomotor development.

Array-CGH demonstrated in the patient a 4q34.1 de novo deletion, encompassing some candidate genes. Further studies are pending and will be discussed.

#### P02.087

##### Case report: Waardenburg syndrome and chromosome 13q deletion

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Waardenburg-Shah syndrome (WS4; MIM 277580) is a disorder of the embryonic neural crest that combines clinical features of Waardenburg syndrome and Hirschsprung disease. The phenotype can be caused by mutation in the endothelin-B receptor gene (EDNRB), in the gene for its ligand, endothelin-3 (EDN3) or in the SOX10 gene.

We report a case of a 3 years old patient with hypotonia, mild psychomotor and speech delay, and dysmorphic features. He was born a term with a normal pregnancy. At one month he suffered of recurrent vomiting caused by hypertrophic pyloric stenosis. During the recovery were found also abnormal values of yGT and transaminases. The ERCP and biopsy did reveal any hepatic involvement neither biliary obstruction. Moreover, a NMR was performed because of hypotonia and mental retardation and corpus callosum agenesis was evidenced. An ABR exam revealed also a monolateral sensorineural hypoacusia. The patient presents with heterochromia irides, wide nasal bridge, short nose with smooth philtrum, mild retrognathia and legs' hypopigmented skin patches. For the clinical signs a syndrome was suspected and a standard karyotype was performed. The analysis indicate an interstitial deletion of the long arm of chromosome 13. To better define the chromosomal anomaly we performed a CGH Arrays. The result showed the presence of a  $12.5 \pm 1.9$  Mb deletion at 13q31.1 à q21.32 region. The deletion involves the EDNRB gene, linked to WS4. Because of the presence of the other signs that don't fit with WS4 phenotype, we are trying to correlate the clinical features with the deleted genes.

#### P02.088

##### Prenatal detection of an interstitial 10p deletion: case report

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Background: Partial 10p monosomy is a rare chromosomal abnormality, with approximately 50 reported patients. Phenotypic spectrum of patients with chromosomal rearrangements of that region is wide. A critical haploinsufficiency region for DGS/VCFS (resembling Di George syndrome) has been described in certain patients with 10p13p14 deletion. This region encompasses two different loci, HDR1 (hypoparathyroidism, deafness, renal dysplasia) and DGCR2 (cardiac defect, T cell deficiency), but the phenotype-genotype correlation is not clear. No cases of deletions more proximal than these loci have been reported.

Case report: We report a prenatally diagnosed interstitial deletion of 10p in a male patient. Antenatal karyotype was undertaken after cerebral ventriculomegaly and ventricular septal defect (VSD) were detected. An interstitial 10p deletion was diagnosed, with normal FISH of DGCR2 locus. Few days later, the patient was born, at term, with normal growth parameters. At 13 months of age, growth was on -1.5 SD with postnatal microcephaly. He had delayed psychomotor development, axial hypotonia and peripheral hypertension. Facial dysmorphia included bilateral ptosis and epicanthus, anteverted nares, dysplastic ears and Cupid's bow upper lip. Severe bilateral deafness, perimembranous VSD, bilateral cryptorchidism, chorio-retinal coloboma and toe

camptodactyly and syndactyly were noted. Brain MRI showed ventriculomegaly, cerebellar hypoplasia and bilateral anterior optic nerve hypoplasia. Array CGH was performed after birth in order to determine the precise 10p deletion, and revealed a proximal deletion: 10p11.2-p12.1.

Conclusion: We report a patient with prenatally detected unreported proximal interstitial 10p deletion, with multiple congenital abnormalities, including conotruncal cardiac malformation, deafness, cerebral malformations and specific dysmorphia.

#### P02.089

##### Molecular cytogenetic characterization of an interstitial 14q24q32 deletion in a girl with corpus callosum hypoplasia

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Interstitial deletions of 14q including the q31 region are uncommon. Only 15 cases were reported.

We report on a 3 year-old Tunisian girl with an interstitial deletion of the long arm of chromosome 14 diagnosed by standard karyotype.

The girl presented with dysmorphic features, developmental delay and hypoplasia of corpus callosum. Using Fluorescent In Situ Hybridization technique (FISH), we characterized the deletion boundaries corresponding to the Bacterial Artificial Chromosomes (BAC): RP11-501I4 and CTD2348N10.

The karyotype was interpreted as 46,XX,del(14)(q24.3q32.2) covering nearly 24Mb.

Our patient shares with the previously reported 14q31 deletions some similar features such as microcephaly, hypotonia, ears abnormalities, strabismus, hypertrichosis and carp mouth.

She presents a corpus callosum hypoplasia on cerebral MRI which the first time reported in association to such deletion.

However, the hypoplasia of corpus callosum is described only in our patient. We propose that the deleted region in our patient could present a candidate gene for this cerebral abnormality.

#### P02.090

##### A 0.43 Mb region within the 1q44 commonly deleted in three patients with microcephaly and agenesis of the corpus callosum

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Complex congenital phenotypes involving mental retardation and developmental delay frequently result from structural genome aberrations such as interstitial or subtelomeric microdeletions. Although some of these aberrations may lead to recognizable phenotypes, a high degree of phenotypic variability often complicates a comprehensive clinical and genetic diagnosis. We describe three patients who share developmental delay and mental retardation, including retarded development and limited speech abilities, hypotonia, febrile seizures or epilepsy, hypertelorism, an abnormal corpus callosum and other CNS anomalies. By high resolution oligonucleotide and SNP array-based segmental aneuploidy profiling we detected in chromosomal region 1q44 three overlapping deletions of 1.250, 4.210 and 9.436 Mb in size. The three patients share a 0.43 Mb interstitial deletion, which contains the FAM36A, HNRPU and the EFCAB2 genes. This region does not contain AKT3 and ZNF238, two previously proposed candidate genes for microcephaly and agenesis of the corpus callosum. Therefore, we conclude that the HNRPU gene, which is deleted in all deletion 1q44 patients reported thus far, may represent an additional candidate for the core features of the terminal 1q deletion syndrome. Since only one of the two patients with deletions including the ZNF124 gene showed a Dandy Walker Malformation, mere hemizygosity for this gene seems not to be sufficient to cause this anomaly.

**P02.091****Identification of a rare cytogenetic aberration of chromosome 5 identified by fluorescent *in situ* hybridization****L. Losonczi, M. Dobos, J. Szabó, J. Szabolcs, T. Bense, G. Fekete;****2nd Department of Pediatrics, Budapest, Hungary.**

**Introduction:** We report a rare cytogenetic aberration of chromosome 5.

**Case report:** Our patient, after delivered from second, with polyhydramnion complicated pregnancy, had bradycardia and cyanosis. His gestational age was 39 weeks, and the birthweight was 2910 grams. After delivery he had to be ventilated for some minutes. He got antibiotics because of perinatal infection. During feeding he became cyanotic. Echocardiography revealed 10 mm wide second type atrial septal defect. Neurological investigations revealed generalized hypotonia. Other characteristic finding was the baby's strange crying. Some minor anomalies could be detected, such as low and back sitting ears, short palpebral fissure, abnormal shape of the cranium, and clubbed fingers. In the family-history was no abnormality. Because of minor abnormalities and congenital heart defect cytogenetic investigations was done. The cytogenetic investigation with G bands and FISH revealed the duplication of the cri du chat region [46,XY,dup(5)(p15.2 p15.3)]. Parents' karyogram was normal. This type of cytogenetic abnormality of chromosome 5 is rare, 9 cases have been reported until now, with similar clinical and cytogenetic findings. The feeding of the infant was difficult, suffered often from infections, had failure to thrive. Because of the wide ASD the cardiac function became worse, severe pulmonary hypertension developed. He died at the age of 6 months, with symptoms of circulatory insufficiency. **Conclusion:** A rare cytogenetic aberration of chromosome 5 is described, which help to understand the genotype-phenotype correlation in patients with 5p duplication.

**P02.092****A submicroscopic 5q11.2 deletion in a girl with autism, mild mental retardation and mild facial dysmorphism****H. Peeters<sup>1</sup>, A. Crepel<sup>1</sup>, K. Devriendt<sup>1</sup>, P. De Cock<sup>2</sup>, J. Vermeesch<sup>1</sup>, J. P. Fryns<sup>1</sup>;****<sup>1</sup>University of Leuven, Center for Human Genetics, B-3000 Leuven, Belgium,****<sup>2</sup>University of Leuven, Child Neurology Department, B-3000 Leuven, Belgium.**

We report on a female patient with autism spectrum disorder (ASD), mild mental retardation, mild facial dysmorphism and a submicroscopic 5q11.2 deletion detected by 1-Mb array CGH. In addition, a 2Mb maternally inherited duplication on Xp22.31 (RP11-483M24->RP11-323F16) was detected. Chromosomal imbalances (deletions or duplications) appear to be present in about 25% of patients with syndromic ASD. The deletion in the present patient is approximately 8 Mb in size and is flanked by the clones CTD-2276024 and RP11-210O14. Interestingly, a similar but possibly slightly smaller deletion on has previously been described in a boy with profound speech delay, obsessional play and echolalia (K Prescott et al. 2005). In contrast to the patient we describe, this boy presented additional malformations like a cardiac defect (tetralogy of Fallot), a bifid uvula, velopharyngeal insufficiency and short stature. Since autistic behaviour is the only consistent finding in 2 patients with a similar deletion 5q11.2, the location of this deletion may identify a gene that is implicated in autism spectrum disorders. Furthermore our observation illustrates that array-CGH should be considered as an essential aspect in the genetic analysis of patients with syndromic ASD.

**P02.093****Non acrocentrics sSMC: thirteen new cases****M. Santos, A. Escalona, C. Hernando, C. Fuster;****Universitat Autònoma de Barcelona, Bellaterra, Spain.**

**Context** Small supernumerary marker chromosomes (sSMC) are common findings in Prenatal and Postnatal Diagnosis. So far, the genetic origin of these sSMC usually remained unknown. Recently the use of several technologies based on FISH has allowed an important progress toward this goal.

**Objective** To characterise 50 sSMC detected in unrelated patients with constitutional genetic disorders, mental retardation or infertility.

**Methodology** G-banding, HR-CGH and FISH (M-FISH, BACs)

**Results** Acrocentric sSMC were detected in 74% (37/50) of cases. Inv dup(15) was the most commonly detected sSMC followed by inv dup(13 or 21). Non acrocentric sSMC were detected in the rest of cases (26%, 13/50). sSMC derived from chromosomes 2, 3, 4, 7, 8, 9, 11, 17,

18 (three cases), 20 and X were also identified and two of them were neocentric (those derived from chromosomes 3 and 20). In two cases, we detected at least 4 sSMC derived from the same chromosome but with different morphologies. In one they derived from chromosome 9 and in the other they derived from chromosome X. In general, we found according with the literature that the presence of euchromatin in the *de novo* sSMC is linked with an abnormal phenotype.

**Conclusion** The use of combined molecular cytogenetic techniques revealed that the incidence of non acrocentric sSMC is probably higher than expected.

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**P02.094****Familial chromosomal rearrangement with an extra micro derivative 22 compensating a 22q11.2 deletion****J. Nevado<sup>1,2</sup>, M. A. Mori<sup>1,2</sup>, L. Fernández<sup>1,2</sup>, P. D. Lapunzina<sup>1,2</sup>, B. García<sup>3</sup>, M. L. De Torres<sup>1,2</sup>;****<sup>1</sup>Hospital Universitario la Paz, Genética Médica, MADRID, Spain, <sup>2</sup>CIBERER, Madrid, Spain, <sup>3</sup>Hospital Universitario Príncipe de Asturias, Bioquímica Clínica, Unidad de Genética, Alcalá de Henares (MADRID), Spain.**

We described a non-consanguineous couple (33 year-old) that underwent amniocentesis for prenatal cytogenetic studies (in the 15<sup>th</sup> gestational week) due to the man is carrying an unusual rearrangement with an extra micro-chromosome, which renders a high probability to have cases of 22q11.2 deletion syndrome in the family. His karyotype is: 46, XY [22%] /47, XY, + mar [78%]. ish del (22) (q11.2 q11.2) (D22S987E-D22S976E-)/ ish del (22) (q11.2 q11.2) (D22S987E-D22S976E-), + mar. ish der (22) (D22S987E-D22S976E+).

The prenatal cytogenetic study, including FISH, in amniotic fluid (43 cells studied from three independent cell cultures) and cord blood (100 cells studied) showed a deletion in region 22q11.2: 46, XY. ish del (22) (q11.2 q11.2) (D22S987E-D22S976E-). No trace of an extra micro-chromosome was found. In the Genetic counselling clinics the couple was told about the consequences of 22q11.2 deletion syndrome and they decided termination of the pregnancy.

An important remark in this family is that, members who are carrying the extra chromosome are phenotypically normal because they are chromosomally balanced at this region. Thus, it must be important to rule out the 22q11.2 deletion, using FISH and/or MLPA, in members of the family with apparently normal number of chromosomes.

**P02.095****Autistic features with speech delay in a girl with an ~1.5 Mb deletion in 6q16.1, including FUT9 and GPR63****K. W. Derwinska<sup>1,2</sup>, J. Bernaciak<sup>1</sup>, E. Obersztyn<sup>1</sup>, E. Bocian<sup>1</sup>, P. Stankiewicz<sup>1,2</sup>;****<sup>1</sup>Institute of Mother and Child, Warsaw, Poland, <sup>2</sup>Dept. of Molecular & Human Genetics, Baylor College of Medicine, Houston, TX, United States.**

Recent studies have shown that up to 40% of the apparently balanced reciprocal chromosome translocations in patients with abnormal phenotype can be accompanied by a chromosome imbalance. We present a 10-year-old girl with mild mental retardation, abnormal EEG, and autistic behavior, who began to speak at 5 years. She had no dysmorphic features and her brain MRI was normal. Karyotype analysis revealed a *de novo* apparently balanced translocation t(6;14)(q16;q22). Whole genome array CGH analysis with ~385,000 oligonucleotide probes (NimbleGen) identified an ~1.48 Mb deletion in 6q16.1. FISH with BAC clones mapping within and directly flanking the deleted segment showed that the deletion arose at the translocation breakpoint. Interestingly, autism has been linked previously to chromosome 6q16. The deleted segment harbors FUT9, GPR63, FHL5, KLHL32, c6orf66, and AK091365 and two predicted genes c6orf167 and KIA0776. GPR63 is expressed almost exclusively in the brain and encodes a G-protein-coupled receptor for sphingosine 1-phosphate. The fucosyltransferase 9 gene, FUT9, is highly conserved among humans, mice, rats, and hamsters and is highly expressed in brain during embryogenesis. FUT9 is considered to be involved in cell-cell interactions, differentiation, and neurodevelopmental processes. Our data confirm previous observations that copy-number variation is a significant factor responsible for autistic spectrum behavior and speech delay.

**P02.096****Delineation of a Critical Region on Chromosome 18 for the del(18)(q12.2q21.1) Syndrome**

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Array CGH has been instrumental in the identification of submicroscopic chromosomal aberrations leading to mental retardation and congenital abnormalities (MR/MCA), the delineation of new microdeletion syndromes and the identification of genes implicated in MR/MCA syndromes. An important step prior to assigning particular phenotypic features to particular genes is the delineation of critical regions for these specific clinical features. To this purpose, smaller interstitial deletions can be particularly important. In some syndromes, haploinsufficiency of a single gene appears to be responsible for most of the phenotypic features, as is the case for the EHMT1 gene in the 9q34 subtelomeric deletion syndrome. In contrast to distal 18q deletions, proximal interstitial deletions encompassing chromosome band 18q12.3 and parts of neighboring bands (q12.2→q21.1) have been reported less frequently. Up to now, 24 patients carrying such deletions were described. Accurate genotype-phenotype correlations are only possible for those cases for which breakpoints have been molecularly defined.

Here we describe a boy with a submicroscopic deletion of less than 1.8 Mb of subband 18q12.3. The phenotypic features of the proband correspond well with those observed in patients with larger cytogenetically detectable deletions encompassing chromosome band 18q12.3. The deletion has been characterized at the molecular level which is important for defining small regions and eventually specific genes associated with specific phenotypic features. The deletion enabled us to define a critical region for the following features of the del(18)(q12.2q21.1) syndrome: hypotonia, expressive language delay, short stature and behavioral problems.

**P02.097****A novel sex determining locus in a 46,XX-SRY negative sex reversal patient carrying a t(8;19)(q22.1;q13.1)**

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The sex determining genes SRY and SOX9 are required to initiate and maintain testicular development. However, genetic interactions controlling the earliest steps in gonadal development remain poorly understood. The molecular abnormalities underlying a high proportion of XX maleness remain undiscovered. Using of high density SNP array in sex reversal patients could be useful to characterize the etiology of the abnormal gonadal development and provide new molecular insights into the normal regulatory network in the testis and ovary development. We performed SNPs microarray genotyping (Affymetrix geneChip 500k) to investigate homozygosity mapping and LOH in a nuclear family (mother, father, siblings) of a 46,XX-SRY negative male carrying a t(8;19)(q22.1;q13.1). In addition we analyzed 10 sporadic SRY negative XX male. The results were also compared with the International HapMap. The analysis of the break points in the patient revealed in chromosome 19 three intervals with 59, 89, 25 and 15 homozygous SNPs and three regions containing 83, 73 and 57 LOH SNPs located in chromosome 8. Analysis of the data revealed four sex determining genes candidates, we present and discuss the genomic data and the analysis performed in order to identify a novel locus involved in the testicular development in humans.

**P02.098****Prenatally diagnosed recombinant offsprings resulting from adjacent-1, adjacent-2, 3:1 segregation modes of reciprocal translocations:fish and cytogenetic studies**

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Balanced translocation carriers can have recombinant offsprings due to adjacent-1, adjacent-2, 3:1 and 4:0 segregation modes. Here, we report three prenatally diagnosed recombinant fetuses resulting from balanced parental translocations.

Case1: Amniocentesis was performed because of bilateral choroid plexus cysts and hyperechogenic bowel. Fetal karyotype showed a de-

rivative chromosome 1p[46,XY,der(1)t(1;?)(p36,1;?)] which was paternally originated:46,XY,t(1;8) (p36,1;p21,3)]. FISH confirmed translocation between chromosomes 1 and 8; fetal karyotype was interpreted as partial monosomy 1p/partial trisomy 8p. Adjacent-1 segregation mode was suggested. Parents decided to terminate the pregnancy. Fetus presented facial dysmorphism, limb abnormalities, cerebellar hypoplasia, ventriculomegaly, bilateral choroid plexus cysts, lung defects.

Case2: Amniocentesis was performed due to fetal abnormalities: cleft lip/palate, tetralogy of fallot, ASD, single artery/vein of umbilical cord. Cytogenetics revealed unbalanced chromosomal translocation:46,XY, der(15)t(10;15) (q22.2;q11.2), paternally derived [46,XY,t(10;15)(q22.2 ;q11.2)]. Adjacent-2 segregation mode was suggested. FISH analysis confirmed der(15)t(10;15). Pregnancy ended spontaneously after amniocentesis. The fetus couldn't be investigated.

Case3: Amniocentesis was performed because of positive maternal serum triple screening. Cytogenetics revealed a supernumerary chromosome(47,XY,+mar). Parents were karyotyped. The mother showed balanced reciprocal translocation:t(2;14)(p23;q23). Supernumerary chromosome was identified as part of chromosome 14 (FISH analysis). Fetal karyotype was interpreted as:47,XY,+der(14)t(2;14)(p 23;q23)mat; partial trisomy 2p/partial trisomy 14q due to 3:1 segregation was suggested. Pregnancy was terminated. Extensive clinical evaluation showed fetal dysmorphism and open cranial sutures.

**P02.099****Male pseudohermaphroditism: a case report**

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Male pseudohermaphroditism is characterized by the presence of 46, XY karyotype, exclusively male gonads and ambiguous or female external and/or internal genitalia, caused by incomplete virilization during prenatal life. We report the case of an infant, in whom physical examination showed ambiguous external genitalia and some dysmorphic features. Cytogenetic investigation was performed for the correct assignment of the gender and chromosome analysis in blood lymphocytes revealed male genetic sex. The ambivalent aspect of the external genitalia, the gonads that are exclusively testes together with the result of the cytogenetic analysis showing male genetic sex, led to the diagnosis of male pseudohermaphroditism. A multidisciplinary approach involving pediatricians, specialists in the field of endocrinology, genetics, surgery and psychiatry is necessary in order to reach a prompt and correct diagnosis and treatment. In conclusion, careful examination of the external genitalia of every newborn should be systematic. Early diagnosis of a disorder of sexual development is of great importance as is the appropriate gender assignment. The most important decision will be the choice of sex assignment, which will depend on many complex factors. Birth registration should be postponed until the diagnostic evaluation enables the most appropriate choice of sex for rearing. The clinical management will help the child and the family deal effectively with this disorder.

**P02.100****PCR based technique for sex selection in cattle**

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Embryo sexing is an important way 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 and which also contains various internal repeated sequence. S4 is localized on the long arm of the Y chromosome in the region of ZFY genes.

Aim: The objective of this study is to identify embryo sexing by a simple and precise PCR method.

Materials and Methods: Genomic DNA was extracted from the whole blood samples. Two sets of specific primers were designed

Result: By this PCR based methods we could differentiate between female and male genomic DNA.

Discussion: With this technique we can distinct males from females. This method has the potential to be employed for embryonic sex selection.

**P02.101****Case report: A case of a rare translocation of SRY region of the Y chromosome to the short arm of the X chromosome**

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The 46, XX karyotype male syndrome is a rare sex chromosome disorder occurring in less than 1 in 25 000 individuals. It mostly results from unequal crossing-over between the X and Y chromosomes during meiosis. Here we report a two weeks old boy with bilateral cryptorchidism and penile hypospadias. Chromosomal analysis revealed a 46, XX karyotype and the FISH test showed the presence of the SRY region of the Y chromosome translocated to the short arm of the X chromosome. SRY gene located in this region is the main gene responsible for gonadal differentiation in the male and it is essential for the normal development of male genitalia. This report confirms the value of karyotyping and FISH analysis in the cases of ambiguous genitalia.

**P02.102****Mosaicism characterization in male with 46,XX SRY positive karyotype**

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46,XX "male syndrome" is rare chromosomal disorder in human that occurs at about 1 in 20 000 - 25 000 males. The SRY gene, located at p11.3 chromosome Y, plays a key role in human sex determination and is responsible for the reproductive system morphogenesis. Most 46,XX male individuals with normal genitalia and karyotype 46,XX are SRY positive. This chromosomal aberration arises due to an unequal recombination between Xp and Yp terminal region during paternal meiosis.

Here we present a case of an infertile (azoospermia) 32 years old male, who has normal masculinization of the external genitalia. A standard cytogenetic study revealed the karyotype 46,XX in 25 analyzed metaphases.

FISH analysis using LSI SRY probe Spectrum Orange (Vysis) showed the presence of single SRY gene signal in 50 metaphases studied. The subsequent FISH using chromosome X WCP probe Spectrum Orange (Vysis) has demonstrated the localization of SRY signal on p-arm of chromosome X, and constitutional karyotype abnormality was interpreted as 46,XX.ish der(X)t(X;Y)(p22.3;p11.3)(SRY+). Furthermore the analysis of 184 metaphases has allowed to ascertain the mosaic status of the karyotype in lymphocytes: 45,X.ish (wcpXx1)[4]/47,XXX.ish (wcpXx3)[3]/48,XXXX.ish (wcpXx4)[1]/46,XX.ish (wcpXx2)[176].

The results obtained demonstrate the advantage of FISH for detecting low-grade mosaicism in patients with anomalies of sex chromosomes.

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**P02.103****Clinical, Cytogenetic and Molecular Cytogenetic Study of Three XX Male Cases**

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46,XX male is a rare disorder that occurs at about 1 in 20,000 males. It is due to accidental recombination between the short arm of the Y chromosome and the short arm of the X in paternal meiosis. This results in translocation of the SRY gene from the Y to the X chromosome. Aim of this study is to report and highlight the value of cytogenetics and (FISH) analysis for males with 46, XX Karyotype, since the phenotype does not always correlate with the presence or absence of Y sequences in the genome.

In this report we present clinical, cytogenetic and molecular cytogenetic data of three patients referred to human cytogenetics department from Clinical genetic department. Two patients presented with infertility with normal male external genitalia and one patient was presenting with ambiguous genitalia. Ultrasonography revealed no müllerian derivatives in the three patients.

Cytogenetic results for the three cases revealed 46, XX karyotype. FISH analysis showed the presence of SRY gene on the short arm of X chromosome in cases numbers 1 & 2 and its absence in case number 3.

Analysis of these results illustrated that combined conventional cytogenetic and FISH techniques are essential for accurate diagnosis and

proper genetic counseling. Therefore we recommend performing molecular cytogenetic (FISH) in cases of male infertility or male genital ambiguity.

**P02.104****A 14 bp deletion in the sry gene associated with xy sex reversal**

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Sex-reversed XY females can arise from different mechanisms. About 15% of the XY females with pure gonadal dysgenesis are found to have mutations in the testicular determinant gene SRY (Sex Reversal on chromosome Y), specially affecting the high mobility group conserved region HMG box. The SRY gene is located at Yp11.31 and induces male sexual differentiation in human embryos from the 6<sup>th</sup> week of gestation. The failure of this process causes gonadal dysgenesis in complete or incomplete form, depending on the presence or not of testicular tissue remnants. Here we present a female 15 years old with no pubertal development, hypergonadotropism, obesity and diabetes, showing a 46,XY karyotype. The existence of the SRY gene was confirmed by PCR. Sequencing of SRY revealed a deletion of 14 nucleotides in the coding sequence of the gene (2188delAAAGCTG-TAACTCT), located in the 5' region upstream of the HMG box. The deletion of these nucleotides originates a stop codon that will give rise to a severely truncated protein. This defective SRY protein lacks the entire DNA-binding HMG domain and will therefore most likely be non-functional. Only four different deletions have been previously identified in the SRY gene (HMGD database) associated to XY sex-reversal.

**P02.105****Molecular analysis of SRY identifies familial and de novo mutations in 46,XY females with different phenotypes of gonadal dysgenesis**

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In human males, normal testicular determination is firstly triggered by the action of the SRY protein, which is encoded by a single exon-gene (SRY) located on Yp11.3. SRY has a high-mobility group (HMG) domain, which is able to bind and bend DNA. *De novo* point mutations in SRY that arise mainly in the HMG box domain, were identified in 46,XY female patients with complete gonadal dysgenesis (CGD). However, even very rarely, familial SRY mutations were already described. Here, we describe eight 46,XY female patients with mutations in the SRY gene, seven with CGD and one with ambiguous external genitalia. Molecular studies were performed using four sets of primers, from nucleotides -323 to +950 of the SRY. PCR amplified DNA fragments were analysed by SSCP or DHPLC, and subsequently sequenced when appropriate.

In two patients with CGD, a microdeletion including all SRY coding region was found. In the remaining six patients the following point mutations were detected: c.53G>A(p.S18N); 89G>T(R30I); c.169C>T(E57X); 224G>T(p.P63A). While the last two, were not described before, and are *de novo* mutations, c.53G>A(p.S18N) and 89G>T(R30I) were also found in both patient's fathers. 89G>T(R30I) was also detected in a first degree cousin (phenotypically normal) of one of the female patients with CGD. None of the above mutations were found in more than 100 phenotypically normal unrelated males. We evidence, that while some SRY mutations may be the cause of complete gonadal dysgenesis, others are associated with phenotypic heterogeneity, supporting the existence of modifier genes implicated in sex determination.

**P02.106****Aneuploidy of chromosome 21 in the Alzheimer's disease brain**Y. B. Yurov<sup>1,2</sup>, I. Y. Iourov<sup>1,2</sup>, S. G. Vorsanova<sup>1,2</sup>, T. Liehr<sup>3</sup>;<sup>1</sup>National Research Center of Mental Health, RAMS, Moscow, Russian Federation, <sup>2</sup>Institute of Pediatrics and Children Surgery, Rosmedtechnologii, Moscow, Russian Federation, <sup>3</sup>Institute of Human Genetics and Anthropology, Jena, Germany.

Apart from single gene mutations, Alzheimer's disease (AD) is suggested to be associated with mosaic aneuploidy of chromosome 21. This hypothesis has been repeatedly supported by studies of AD blood lymphocytes and skin fibroblasts. However, the diseased brain has not been directly studied. We have performed molecular cytogenetic studies by interphase chromosome 21-specific multicolour banding on cells of AD brain samples derived from the cerebral cortex, hippocampus and cerebellum. The analysis was performed through evaluation of about 14000 interphase nuclei. Control samples exhibited mean rate of aneuploid cells to be about 0.7% (all brain areas). The average rate of aneuploidy achieved 4% in the AD hippocampus, 3.5% in the AD cerebral cortex, and 0.9% in the AD cerebellum. Aneuploidy manifested more frequently as chromosome 21 monosomy, but the proportion of cells with additional chromosome 21 was also significant. The monosomy-to-trisomy ratio was about 2:1. AD brain exhibited significant increase of chromosome 21 aneuploidy in the hippocampus and cerebral cortex. Interestingly, these two areas are known to be more affected by neurodegenerative processes featuring AD. Together, this suggests a correlation between molecular cytogenetic and neuropathology data. We hypothesize that increased aneuploidy rates in the AD hippocampus and cerebral cortex are the results of mitotic errors during adult neurogenesis. Our data shows that specific areas of the senescent brain can be selectively affected by acquired aneuploidy. Aneuploidization of specific brain areas is, therefore, a genetic process that is likely involved in the pathogenesis of AD.

**P02.107****Evaluation of micronuclei and nondisjunction frequencies in workers exposed to internal plutonium by cytokinesis-blocked binucleated cells assay**

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Damage or dysfunction of any component of mitotic segregation complex by mutagens can lead to mistakes in chromosome number. One of the most reliable methods of aneugenic influence detection is FISH-analysis of cytokinesis-blocked binucleated cells. Detection of micronuclei in these investigations is an indicator of clastogenic effects. We have tested abilities of this approach on a group of Siberian Chemical Plant (SCP) workers exposed to internal plutonium. We have formed groups of exposed individuals - SCP workers with incorporated plutonium (10-276 nCi) - 70 individuals, and control set - people living in Seversk and not exposed of nuclear-chemical agents - 50 individuals. In preliminary analysis we have demonstrated that missegregation frequencies for chromosomes 7 and 12 was 0.2% for each chromosome in cells of 27 SCP workers, and 0.1% and 0.075% in 8 individuals from control group, respectively. Significantly increased frequency of nondisjunction in exposed group was noted for chromosome 12 only ( $p=0.03$ ). However, tendency to increase of missegregation of chromosome 7 ( $p=0.06$ ) was observed. Significant differences in frequencies of micronuclei in exposed and control groups (0.77 and 0.74 %, respectively) were not found. Since we have carried out only a preliminary study of abnormal chromosome number frequency it is early to make conclusion about aneugenic influence of incorporated plutonium. However, we can suggest that this approach is sufficiently informative and sensitive for frequency detection of abnormalities which lead to genomic mutations. Subsequent analysis of segregation abnormalities provides some clues to the quantitative features of aneuploidy induction by internal plutonium.

**P02.108****Cytogenetic effects of doxazosina a alpha-blocker antihypertensive drug: sister chromatid exchanges and micronuclei frequencies before and after pharmacological treatment**I. Arrieta Saez<sup>1</sup>, O. Peñagarikano<sup>2</sup>, M. Télez<sup>1</sup>, M. Barasoain<sup>1</sup>, I. Huerta<sup>1</sup>, J. Ramírez<sup>1</sup>, B. Criado<sup>3</sup>, P. Flores<sup>4</sup>, A. González<sup>5</sup>;<sup>1</sup>Dpto Genética, Antropología Física y Fisiología animal Facultad de Ciencia y Tecnología, Bilbao, Spain, <sup>2</sup>Emory University School of Medicine, Atlanta, GA, United States, <sup>3</sup>High School Da Maia, CESPU, Porto, Portugal, <sup>4</sup>Dpto de enfermería, Escuela de Enfermería, Bilbao, Spain, <sup>5</sup>Dpto Medicina Interna, Facultad de Medicina, Bilbao, Spain.

Arterial hypertension is a health problem representing one of the most frequent diagnoses in the population at large in terms of prevalence and incidence. Drug treatment of hypertension may last for decades. Long-term treatment require documentation of long-term safety and efficacy, including sensitive indices of genotoxic damage.

In our previous works Sister Chromatid Exchange (SCE) and Micronuclei (MN) assays were applied to assess the genotoxicity of atenolol a beta-blocker antihypertensive drug and nimodipine a calcium antagonist antihypertensive in culture peripheral lymphocytes of treated patients and control individuals. The results showed no genotoxic effect of nimodipine, however a statistical significant increase in the frequency of MN was detected in patients treated with atenolol. Application of FISH with an alploid stellate probe revealed also a statistical significant higher percentage of centromere positive MN.

We report here our data from a study of the genotoxic potential of Doxazosina. The pharmacological application of the antihypertensive Doxazosina is due to their ability to blocker alpha receptors. This antihypertensive was tested for its ability to induce SCE and MN in cultured human peripheral blood lymphocytes of patients before and after pharmacological treatment, so that the patient is control himself.

The results of the study revealed that the frequency of SCE did not show significant differences. However a statistical significant increase in the frequency of centromere positive MN was detected, thus indicating more frequent involvement of aneuploidy phenomena in the origin of Doxazosina-induced MN.

**P02.109****Chromosome instability in the ataxia telangiectasia cerebellum**I. Y. Iourov<sup>1,2</sup>, S. G. Vorsanova<sup>1,2</sup>, T. Liehr<sup>3</sup>, A. D. Kolotil<sup>2</sup>, Y. B. Yurov<sup>1</sup>;<sup>1</sup>National Research Center of Mental Health, RAMS, Moscow, Russian Federation, <sup>2</sup>Institute of Pediatrics and Children Surgery, Rosmedtechnologii, Moscow, Russian Federation, <sup>3</sup>Institute of Human Genetics and Anthropology, Jena, Germany.

Ataxia-telangiectasia (AT) is a chromosome instability (CIN) syndrome associated with progressive neuronal degeneration affecting exclusively the cerebellum. Molecular basis of cerebellar neurodegeneration in AT is unknown, but hypothesized to be closely linked to the loss of genome integrity in the cerebellar neural cells manifesting as CIN. Here, we monitored CIN in the AT brain by interphase chromosome-specific multicolour banding allowing analysis of interphase chromosomes in their integrity. We found stable 2-4-fold increase of stochastic aneuploidy involving whole chromosomes 7 and 14 in the cerebellum of the AT brain as to controls. Additionally, dramatic 5-40-fold increase of CIN involving chromosome 14 (aneuploidy (including supernumerary rearranged chromosomes) and non-random chromosome breaks) as to other brain areas has been revealed. The latter was found to progress with the age of patients. Paradoxically, the longevity of AT patients was in direct proportion to CIN levels in the cerebellum. We hypothesize that aneuploidization and acquired CIN may result from autoactivation of aberrant processes triggered in response to progressive neuronal degeneration. Newly generated neural cells, being genetically abnormal, nevertheless, partially substitute the loss of cells in the cerebellum and play an ameliorative role in stabilization of the diseased brain homeostasis. We suggest CIN in the AT cerebellum to be involved in processes that mediate either neurodegeneration or endogenous neuroprotection. The way CIN is progressed, can be, therefore, proposed as definitive for the course of neurodegeneration and for cerebellar dysfunction in AT patients.

**P02.110*****CSMD3, a candidate gene for autism found in two patients with autistic disorder and balanced translocations***

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The study of chromosomal rearrangements associated with abnormal phenotypes has proven to be a powerful method for the identification of disease-related genes. Recent studies estimated a rate of 3-5% of cytogenetic abnormalities involving many different chromosomes in autistic spectrum disorders (ASDs). We report here on two unrelated males with *de-novo* translocations, autistic behaviour and psychomotor delay. They carry balanced chromosome translocations, respectively a t(5;8)(q14.3;q23.3) and t(6;8)(q13;q23.2). A detailed physical map covering the regions involved in the translocations was constructed and FISH analyses were carried out using BAC clones mapping on chromosomes 5q14.3, 6q13 and 8q23. We fine mapped the two translocation breakpoints on chromosomes 8 identifying their positions within a short 5 Mb genomic region. The results of these analyses showed that breakpoints on chromosomes 8 in both patients do not interrupt any known gene but both map in a region containing the *CSMD3* gene. No genes were interrupted on chromosomes 5q14.3 and 6q13. Taking into account that *CSMD3* is expressed in fetal and adult brain, our observations suggest that this gene is a good candidate for the pathogenesis of ASDs. To exclude an over-expression or under-expression of *CSMD3* leaded by the rearrangement, we verified the expression of this gene in the leukocytes of the translocated patient by RT-PCR, but any transcript was detected. Future studies, including mutational analysis of *CSMD3* in a large number of patients affected by autism and behavioural studies on a *Csmd3* knockout mouse, will be necessary to confirm or decline this hypothesis.

**P02.111*****Cytogenetic study of the BPES cases***

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Blepharophimosis ptosis epicanthus inversus syndrome (BPES) is a rare genetic disorder with autosomal dominant pattern of inheritance with a frequency of 1 in 50,000. Patients with BPES have a combination of congenital anomalies as small palpebral fissures, epicanthus inversus, low nasal bridge and congenital ptosis. Other features like microphthalmos, anophthalmos, microcornea, hypermetropia, divergent strabismus, high arched palate, cup shaped ears, mental retardation and infertility in females has been reported. BPES has been categorized into two types: type I associated with primary ovarian follicle and type II involves only eye malformation in both sexes. Penetrance is 100% in type I (transmission by males only) and 96.5% in type II (transmission through both sexes). Review of reported cases concluded that a locus for eyelid development is situated at 3q. Various reports linked the deletion in 3q21, 3q22, 3q23, 3q24, 3q25 and translocations t(3;7)(q26-qter:q+), t(X;3)(p22:q21), t(3;8)(q23:p22.1) to the BPES. We analyzed 10 cases of BPES by GTG banding. Six were males with mean age of 24 years and four were females with mean age of 27 years. Different deletions and translocation of chromosome 3 has been linked with the BPES but there is no case reported with 3q26-28 and 5q31.3-33.2 deletions which we found in two cases. We also found 3qter deletion which has already been linked to the BPES. These findings represent severe manifestation of the disease. BPES is a heterogeneous entity, and evaluation and counseling of affected individuals should be undertaken with caution.

**P02.112*****Are evolutionary chromosome rearrangements triggered by non-random nuclear neighbourhoods?***

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We investigated whether non-random nuclear neighbourhoods may have triggered evolutionary chromosome rearrangements, focussing

on the fusion of human chromosome 2 and the reciprocal translocation t(5;17) in the gorilla.

We analysed the nuclear topology of breakpoint flanking loci in pre- and post-meiotic cells from cryo-sections of Rhesus macaque testis tissue, where the fixation of chromosomal rearrangements is expected to occur. Chromosome 5 and 17 breakpoint flanking BAC clones did not co-localise, but were positioned toward the nuclear centre and at the chromosome territory surface. Both findings can be correlated with their rather gene-dense local genomic environment. The resulting short distances between the two loci may have acted as probabilistic trigger for evolutionary rearrangements of gene-dense genomic regions, even more if these reside exposed at the territory surface. Indeed, *in silico* analysis indicates that the majority of breakpoints in great apes characterized so far reside in genomic environments with elevated local gene density compared to the average of the respective chromosome.

In the light of these results it was surprising to find chromosome 2 fusion point flanking BAC clones localized in the nuclear periphery. Despite this, the two loci were frequently positioned in close physical proximity in Rhesus macaque spermatocytes, and even to a higher degree in spermatogonia.

In conclusion, non-random nuclear positioning of genomic loci during meiosis may have triggered these two evolutionary rearrangements. These triggers may be probabilistic, as shown for the t(5;17), or specific side-by-side arrangements in the case of the fusion of human chromosome 2.

**P02.113*****Chromosomal mosaicism of the skin: A preliminary study***

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**Introduction :** The concept of cutaneous mosaicism has now been proved by numerous studies. It seems likely that the two skin types represent two different genotypes, even though this has been remarkably difficult to prove. Our aim is to describe the neurocutaneous symptoms and skin chromosomal mosaicism spectrum in a series of 14 pediatric patients.

**Patients and Methods:** We have reviewed the karyotype of cultured fibroblasts from 75 pediatric patients, who presented skin lesions suggestive of pigmentary mosaicism, studied in our institution from August 1998 to October 2007. Biopsies were obtained from both hypopigmented and hyperpigmented skin. Conventional chromosome banding studies were performed and karyotyped (ISCN, 1995).

**Results:** We reported 14 patients with skin mosaicism: three boys and eleven girls. Six of them presented numerical abnormalities: three cases with diploid/triploid mosaicism, two patients with a marker chromosome (one of them with persistence of the marker in peripheral blood karyotype); and one patient with a chromosome 20 trisomy and West syndrome. The rest of patients (n=8) had structural chromosomal abnormalities, with different chromosomes involved. All of them had typical linear cutaneous lesions except a two-year-old girl with Pallister-Killian syndrome.

**Conclusion:** There is an association between chromosomal mosaicism and hypopigmentation and hyperpigmentation especially following Blaschko lines. We did not find in all patients direct correlation between a specific chromosomal abnormality and neurological affection. Patients with atypical cutaneous lesions or with neurological symptoms could be candidates to perform a skin karyotype even though blood karyotype is normal.

**P02.114*****Cytogenetic effects in somatic cells of irradiated individuals with developed thyroid gland cancer***

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Essential growth of thyroid gland cancer (TGC) among population of extensive territories of Ukraine, Russia and Byelorussia suffered after "iodine stroke" testifies that thyroid gland pathologies - one of the most expressed health consequences of the Chernobyl accident. Data of

biologic (cytogenetic) indication of radiation injury may specify radiogenic character of TGC. Results of radiation epidemiological and cytogenetic examinations at liquidators of the Chernobyl accident consequences have confirmed the radiogenic character of TGC developed at the group of radiated persons.

Increase in frequency of malformations among endocrine pathologies from 1,4 % in 1993 up to 8,9 % in 2001 (more than in 6,3 times) was registered.

It was revealed the correlation of frequency of chromosomal aberrations - radiation markers (dicentrics and rings) in peripheral blood lymphocytes with radiation dose (correlation coefficient - 6,0) in the remote terms after accident in this group of patients.

Mean value of chromosomal aberrations frequency at group of TGC patients living in territories with high density of radioactive pollution exceeds more than 2 times spontaneous level value.

Assignment to TGC patients thymine preparations is recommended in connection with the radioprotective action registered on level of chromosomal aberrations in somatic cells, and also with the purpose of improvement of quality of patient life based on effective hormonal homeostasis rehabilitation after erosion of thyroid gland.

#### P02.115

#### Individual chromosomal radiosensitivity of human lymphocytes as a parameter of cancer risk

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Cytogenetic methods based on chromosome aberrations analysis (G2-assay) make it possible quantitative estimation of radiation effects on human organism taking into account its individual peculiarities and thus to estimate its IR. The main basis for application of cytogenetic methods in radiobiology are high radiosensitivity of human PBL and formation of stage specific radiation-induced chromosomal aberrations. From the other hand elevated levels of chromosomal aberrations are considered to be the proven markers of cancer development in the calculations of cancer risk after exposure to ionizing radiation. The aim of the presented work was evaluation of chromosomal radiosensitivity of healthy individuals and determination those with the increased susceptibility to radiogenic pathologies.

On the basis of the obtained "stage-effect" and "dose-effect" dependencies for chromosomal aberrations modifications of G2-assay were developed. Analysis (in the first mitosis) of chromosomal aberrations levels induced by G2 irradiation (1,5 Gy) of PBL cultures of 110 healthy individuals revealed their high interindividual variability. The highest differences were registered for chromatid breaks which predominated in the spectrum (CV= 37,5%). Statistical analysis of the distributions of the obtained individual cytogenetic parameters indicated bimodality and make it possible to reveal 12% individuals with increased chromosomal radiosensitivity. Cytogenetic evaluation of individual chromosomal radiosensitivity based on G2-assay has its perspectives in the formation of groups with increased risk of radiogenic cancer developing and its primary prophylactics among healthy population.

#### P02.116

#### Clinical Cytogenetics Study in Pediatric Pathology: A Comparison with Unselected Studies

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We studied 1558 pediatric autopsies. 33.1% had congenital anomalies. Cytogenetic evaluation was successfully performed in 298. Based on clinical criteria, karyotyping was performed in cases of isolated malformations and in cases of two malformations when presenting minor anomalies and in cases of multiple congenital anomalies except in those cases due known monogenic disorder.

The distribution by age was: 142(47.6%) perinatal deaths, 37(12.4%) late neonatal, 87(29.9%) postneonatal, 26(8.7%) preschool age, 3(1%) school age, and 1(0.3%) an adolescent.

We collected blood from superior sagittal sinus, skin and fascia from the rectus abdominis muscle.

We studied 206 cases of isolated malformations and 34 with two malformations and none presented any chromosomal anomaly. In the 256 cases of multiple anomalies were detected 70 with chromosomal anomalies. Among the cases of perinatal death there were 24 chro-

mosomal anomalies, of those 17(70.8%) were autosomal trisomies, 4 (16.6%) were structural anomalies, 2 cases (8.8%) were triploidies and 1 case was a mosaic 46,XY/47,XY +mar. There were 46 chromosomal anomalies among the cases of postneonatal death, of those 39(84.8%) were autosomal trisomies, 5 cases of structural anomalies and 2 cases of mosaics with chromosomal markers.

The best combination for metaphases obtention was fascia and skin. The frequency of chromosomal anomalies was 4.5% (70/1558) very similar to unselected studies, but if we consider only the perinatal deaths was 2.9%(24/835) which is lower when compared with unselected studies ( 5.9%). Will be discussed the results comparing with unselected studies.

#### P02.117

#### Chromosomal mosaicism in spontaneous abortions: a case-control study of 500 consecutive chorionic villus samples by interphase FISH (I-FISH)

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Spontaneous abortions (SA) commonly arise from chromosomal abnormalities, which are usually assessed by standard cytogenetic techniques. This suggests significant underestimation of chromosomal mosaicism and application of interphase molecular cytogenetic techniques (i.e. FISH) for uncovering the true occurrence of mosaics among SA. We have analyzed 500 consecutive chorionic villus samples derived from SA by interphase FISH (I-FISH) with DNA probes for chromosomes 9, 13/21, 14/22, 15, 16, 18, X and Y. Chromosome abnormalities were found in 50% of cases. We detected aneuploidy in 39.6% of samples (aneuploidy of chromosome 16 - 10%; monosomy of chromosome X - 8.2%; polysomy of chromosome X - 5.2%; aneuploidy of chromosome 13 or 21 - 5%; aneuploidy of chromosome 14 or 22 - 4.6%; aneuploidy of chromosome 15 - 3.2%; aneuploidy of chromosome 18 - 2.2%; aneuploidy of chromosome 9 - 1.2%). Polyploidy was found in 10.2% of cases. One case was a chimera with multiple aneuploidies (case reported in Vorsanova et al., 2006). Multiple chromosome abnormalities manifested as aneuploidy involving both autosomes and sex chromosomes was detected in 3.7% of cases. Chromosomal mosaicism (aneuploidy/polyploidy affecting at least 5% of cells) was detected in 23.6% of all cases and 47.6% of cases with chromosome abnormalities. To the best our knowledge, the present set of SA samples is the largest one analyzed by I-FISH. Our data demonstrate that chromosomal mosaicism is highly frequent and provide evidence that chromosomal mosaicism is better to study by I-FISH, rather than alternative approaches. Supported by Philip Morris USA.

#### P02.118

#### Chromosomal translocations in couples with recurrent miscarriage - a retrospective study

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Studies have shown that couples with reproductive loss have a high frequency regarding chromosomal translocations (of 3% for females and 2% for males). This study presents the results of conventional cytogenetic analysis in 700 couples referred to our genetic center. Chromosomal analyses were performed using Giemsa-banding according to standard procedure on peripheral lymphocytes. Each couple has received genetic counseling. Translocations have been identified in 23 cases, 11 in male patients and 12 in female patients. Two Robertsonian translocations trob(13;14) have been found in male patients, in different families and 5 types of balanced translocations, on a number of 9 male patients: t(2;21)(q34;p11.2) one case, t(4;16)(q34;q24) one case, t(2;17)(p22;q21) one case, t(7;15)(q14;q27) one case, t(7;14) one case, t(7;18)(p13;q11) one case, t(1;5)(q23;p12) three cases in one family. In women patients, we have identified one case of Robertsonian translocation, trob(13;22), and 9 different types of balanced translo-

tions in 11 persons: t(1,5)(q42.2;q35.2) one case, t(8;16)(q22.2;q13) one case, t(8;X)(q24.2;q26) one case, t(7;10)(p22;p12.1) two cases, t(8;11)(q22.3;q13.5) two cases, t(5;8)(31.2-ter) one case, t(17;20)(q12;q11) one case, t(6,11,12) one case, t(3;6)(q21;q23.2) one case. The frequency of translocations in our study is similar with those present in other studies, and was almost equal in males and females. Knowing the fact that one person is a translocation carrier confirms the etiology of recurrent miscarriage, and allows us to determine the risk of recurrence. These couples received the recommendation to perform amniocentesis in future pregnancies. Both genetic counseling and cytogenetic analysis should be performed before the application of an assisted reproductive technology.

#### P02.119

#### Identification of chromosomal aberrations in Maruthamalai hills, Coimbatore city, India

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The aim of the study was to identify dwellers risk of irradiation exposure in the Maruthamalai hills, Coimbatore. The present study analyzed levels of chromosomal aberrations, a well-known biomarker of early biological effects and a predictor of cancer risk. Cytogenetic analyze has remained the most suitable assay for the evaluation of the genetic damage induced in somatic cells by clastogens present both in occupational and environmental settings. Present study seems to be novel to use for the evaluation of genetic damage in dwellers of Maruthamalai hills, simultaneously the conventional cytogenetic analysis as well as identify the radiation level by assessing the thermo luminescence dosimetry (TLD). TLD were sent by mail to residents who requested the radiation survey. In the present study 54 experimental subjects were selected and equal number of controls also selected. After signing a consent form, both cases (experimental and controls) provided a blood sample (5 ml) to establish cell cultures. Using the conventional cytogenetic analysis, chromosomal-type and chromatid-type aberrations were observed in some experimental cases. Dicentrics and acentric fragments are unstable types of aberrations but in the present study have not observed any of this aberration in experimental. Finally, present study may conclude that low-doses of ionizing radiation are exposed do not represent by themselves any significant risk of genetic damage as measured by the conventional cytogenetic analysis.

#### P02.120

#### Clinical manifestation of chromosome 2 long arm deletion: report of five cases presentation of four cases

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Terminal and interstitial deletion of the long arm of chromosome 2 belongs to the most common structural chromosomal aberrations. Clinical manifestations of this syndrome comprise: global psychomotor delay, moderate to severe mental retardation with specific facial dimorphism. We report the characteristics of clinical features in five cases of terminal or interstitial deletions in 2q32.2q35, 2q33q34, 2q36, 2q37 and 2q37.3 identified in conventional G banding, fluorescent in situ hybridization (FISH) and High Resolution Comparative Genome Hybridization (HR-CGH) techniques. In one case the deletion of subtelomeric region of chromosome 2 (2q37.3) was added to 2q34q37.2 duplication. A comparison was made of clinical symptoms present in our patients with relevant data concerning other cases of 2q monosomy reported in literature in order to establish an accurate phenotype-genotype correlation and proper genetic counselling of these families.

#### P02.121

#### Chromosome 9: Deletion Duplication of chromosome 9

#### Phenotype karyotype correlations Case Reports brief review

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Chromosome 9 is exhibits marked heteromorphism and pericentric inversion. Deletion of chromosome 9p presents with characteristic phenotype occasionally with overlapping features of Down syndrome. Terminal deletion of 9- was first reported by Alfi et al., Deletion 9p is a rare syndrome. We report here clinical and cytogenetic findings in two cases of deletion 9p and two case of partial trisomy 9. resulting familial translocation (t8p9q)mar and a balanced de novo translocation t(Yq;9p).

The clinical finding is correlated with karyotypes and compared with other reported cases in the literature.

A brief review of literature of deletion duplication of chromosome 9 will be presented.

#### P02.122

#### Combined interstitial duplication 11q22q23 and deletion of 11q24.3: report of a case.

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Background: Detection of abnormal karyotypes with associated clinical manifestations is an important tool for the identification of chromosome regions with genes that confer susceptibility to genetic disorders.

Objective: Identified the extra material present in a *de novo* 46,XY,add(11)(q24.1) karyotype from a child with mental retardation, ocular malformations, mild dimorphic faces, thrombocytopenia and hypogammaglobulinemia.

Methodology: Conventional cytogenetics, High Resolution Comparative Genome Hybridization (HR-CGH), FISH with BAC probes.

Results: The HR-CGH profiles showed the gain of 11q22q23 and deletion of 11q24.3 chromosome bands. Revaluation of G-banded chromosome spreads and FISH with BAC probes across the region confirmed CGH results.

Conclusion: Partial 11q trisomy is unusually in Prenatal and Postnatal Diagnosis. In most cases is associated with 11q22q translocation and a 3:1 meiotic disjunction with 47 chromosomes. The presence of the pure dup (11)(q22q23) in our patient provide an opportunity to delineate the phenotypic features due this partial trisomy, since deletion of 11q24.3 associated phenotypic features are well known.

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#### P02.123

#### Hidden chromosome instability in peripheral blood lymphocytes of unexposed and irradiated persons revealed by means of "G<sub>2</sub> bleomycin sensitivity assay"

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With help of modifying "G<sub>2</sub>-bleomycin sensitivity assay" (treatment of human peripheral blood lymphocytes culture in late post-synthetic phase of mitotic cycle by bleomycin in concentrations 0,05 and 5,00 mcg/ml) the investigation of hidden chromosome instability in 9 unexposed donors and in 32 persons with different intensity of radiation exposure (Chernobyl liquidators including patients with acute radiation syndrome - ARS) had been fulfilled. Main criterion of chromosomes' sensitivity to bleomycin exposure total frequency of chromosome aberrations had been considered. In all examined groups individual levels of chromosome injuries under identical mutagenic exposure varied in wide range and didn't depend on their initial values in intact cultures. In control donors and in 10 liquidators with low radiation exposure the mean-group frequencies of induced chromosome aberrations were quite the same under both testing concentrations of bleomycin. Among control donors and liquidators three by three hypersensitive persons had been identified with aberrations' rates 35,0, 34,0, 16,0 and 34,5, 20,0 19,3 per 100 cells, accordingly that can be considered as genetically caused phenomenon. In 19 ARS patients increased mean-group frequencies of aberrations induced by bleomycin in both concentrations had been revealed - 16,80±0,50 (8,7 - 38,2) and 28,04±0,63 (6,0

- 60.0) per 100 cells accordingly. In this group 13 persons with hidden chromosome instability (from 22.2 till 60.0 aberrations per 100 cells) had been found. The data received permit to assume that high radiation doses even in delayed terms following irradiation can modify inherited human chromosomes susceptibility to mutagen exposure.

#### P02.124

#### Chaotic banding pattern of a chromosome 3 in a patient with mental retardation

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Constitutional complex chromosomal rearrangements (CCRs) are very unusual, and most of them involve more than one chromosome. As far as we know there are only eight published cases having a CCR involving four or more breakpoints within a single chromosome.

Here we present an intrachromosomal CCR in a chromosome 3 having five breakpoints, which was found in a patient without major congenital defects but presenting mental retardation.

The patient was a 30-year-old male, product of the second pregnancy of healthy and non-consanguineous parents. He was born at 42 weeks of gestation by normal vaginal delivery. Few hours after birth, he suffered a hypoxia episode without an apparently cause, that required oxygen therapy. His evolution showed psychomotor delay.

The patient was referred to us for diagnostic evaluation at the age of 30 years, referring mental retardation and a previous karyotype with an inversion in chromosome 3 with breakpoints in p12-q32. We performed a high resolution G-band karyotype and found a chromosome 3 with a chaotic banding pattern. This alteration was "de novo".

Fluorescence *in situ* hybridisation (FISH) with subtelomeric probes 3p/3q showed that they were located at normal position. FISH with WCP3, showed the derivative chromosome 3 homogeneously stained, demonstrating the intrachromosomal nature of the rearrangement. Multicolour-banding FISH was performed and revealed the presence of five breakpoints.

We postulate the different steps that happened to generate the chaotic banding pattern of this CCR.

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#### P02.125

#### Comprehensive Copy Number Variant (CNV) analysis of neuronal pathways genes in mental retardation

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Cryptic copy number variants (CNVs), including deletion and duplication, translocation and inversions, in chromosomes have been identified in about 10% of patients with mental retardation (MR) of unknown origin. The aim of this work is to perform a comprehensive screening of CNVs that contain genes related to neurodevelopment. We selected 364 genes involved in neuronal pathways and used the Database of Genomic Variants to identify genes predicted to be in CNVs (n=75). We designed four Multiple Ligation Probe Amplification (MLPA) assays to detect variations in copy number between patients and controls. We studied 93 children with unexplained MR and normal karyotype and 332 control samples. We discovered 13 CNVs in MR patients, 5 of which were not present in controls. When available, parental samples were also analysed by MLPA to assess the inheritance of the CNV. We found 6 genes located in de novo genomic alterations corresponding to four different genomic loci: 1q42.1 (DISC1 and TSNA), 3p26.1 (GRM7 and CNTN6), 7q31.33 (GRM8) and 17q21.31 (MAPT). The locus on chromosome 17q21.31 has been validated by CGH-array and coincides with the recently described 17q21.31 microdeletion syn-

drome. Some of the regions described here were previously described in other patients with MR. Our results indicate that a considerable proportion of genes involved in neuronal pathways show variability in copy number and that de novo events might be related to the aetiology of MR. Further investigation in larger cohorts of patients should allow a definition of the potential role of genomic variability in MR.

#### P02.126

#### Cryptic deletion Xp21 with the loss of the genes DMD, GK and NR0B1 in a female child with mild psychomotor retardation and stereotypies

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We present the case of a female child at age of 21 months who presented the following clinical features: delayed motor milestones, hypotonia, lethargy during the first months of life, attention deficit, motor limitations (i.e. running difficulties), stereotypies and some autistic features, sleep disturbance, hyperCKaemia. Her psychomotor development improved significantly after the age of 1 year.

By the mean of Array-CGH analysis (BAC-array at an average resolution of 1 Mb) we revealed a de novo deletion of about 4,5 Mb on chromosome Xp21, with the complete loss of the genes DMD (dystrophin), GK (glycerol kinase), NR0B1 (nuclear receptor subfamily 0, group B, member 1) and, probably, of part of the gene IL1RAPL1. The deletion arose on paternal X-chromosome. A random X-inactivation pattern was found.

Clinical-pathogenetics features are discussed, mainly with regard to the early onset of signs of dystrophinopathy in a female patient with an heterozygous deletion of the gene DMD and to the possible phenotypic causal role of the gene IL1RAPL1, whose mutations in hemizygous males has been reported in literature as responsible of some cases of X-linked mental retardation.

#### P02.127

#### Proximal interstitial deletion in the short arm of chromosome 3: a report of a child whose mother carries a balanced reciprocal translocation t(7;14)(p32;q32)

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Proximal 3p deletion is a rare condition. To date, 14 patients have been described. This recognizable phenotype consists of growth retardation, developmental delay, hypotonia and dysmorphic features (platigiocephaly, broad forehead. Broad nasal bridge, low set ears, long philtrum).

We describe a boy with a de novo interstitial deletion at band 3p13p12.3, who presented with a phenotype similar to the previously described cases. However, his mother carries a balanced translocation involving different chromosomes, t(7;14)(p32;q32).

The patient was born at 36 weeks gestation and was diagnosed with hypotonia, feeding difficulties, dysmorphic features and ASD. Brain CT showed partial agenesis of the corpus callosum. G-banded metaphase chromosomes revealed a normal karyotype 46,XY.

At the age of five years, he came for genetic reevaluation. He has psychomotor retardation, severe speech delay and he suffers of seizures. He has dysmorphic features similar to those described in other patients with proximal 3p deletion. CGH microarray analysis was performed using 4685 BAC clones (Signature Genomics Laboratories, Spokane, WA). An interstitial deletion at the short arm of chromosome 3p13p12.3 was identified. FISH analysis, confirmed the microdeletion on the patient's karyotype, but not on the parents' karyotype.

Although the mechanism underlying this cytogenetic event is not understood, we suggest that offsprings of balanced reciprocal translocation carriers, may be more prone to other cytogenetic abnormalities warranting further investigation.

#### P02.128

#### Accuracy of analysis in Cytogenetics

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Cytogenetic tests are undertaken to ascertain a specific syndrome/disorder, exclude a chromosomal cause for multiple miscarriages, define gender, or whether a pregnancy is chromosomally abnormal. For most patients a genetic test is only performed once in a lifetime and it is essential, therefore, that patients receive an accurate result.

A measure of the accuracy of a diagnostic service can be provided through External Quality Assessment (EQA), including the new European EQA scheme, CEQA (Cytogenetic European Quality Assessment) and the National EQA schemes. CEQA has been piloted for the last two years supported by the Eurogentest Network of Excellence. The majority of laboratories participating in these EQA schemes demonstrated satisfactory EQA performance. However, some laboratories did not detect the chromosome abnormality and in rare cases even invented a chromosome abnormality.

An important aspect of cytogenetic analysis is to ascertain the breakpoints involved in a structural rearrangement. However, marked differences in breakpoints were allocated by participating laboratories for the same chromosome abnormality. Information on chromosome breakpoints may be used to initiate additional genetic tests, to ascertain whether a child will be affected with a specific syndrome, to identify a critical region or for gene mapping. This imprecision may have consequences for the validity of any subsequent investigations. The EQA process has also identified significant variation in the extent of interpretation given. Some of the common problems identified through EQA submissions will be presented.

#### P02.129

#### Mosaic partial trisomy of chromosome 8 in a dysmorphic newborn child with multiple anomalies

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We present a case of a dysmorphic newborn girl having congenital anomalies - polycystic kidneys and agenesis of corpus callosum. Anamnestically: the patient was born 4G/3P prematurely at 33. gestation week. Pregnancy duration and results of antenatal investigations were abnormal - polyhydramnion and dysplastic kidneys were diagnosed by ultrasound at 32 gestational week and bone dysplasia was suspected. Birth anthropometry of the patient (209g/47 cm; OFC 32 cm) was according to gestational age, due to respiratory failure she needed artificial ventilation. The patient died at the age of one month. Phenotype of the patient was dysmorphic: low forehead, enophthalmia, bulbous nose, deformed earlobes, bilateral contractures in III fingers and congenital anomalies of brain and kidneys were diagnosed.

Cytogenetical investigations: Rapid chromosomal analysis from peripheral blood showed mosaic result: 46,XX/47,XX,+mar. Additional cytogenetical analysis by G-banding and FISH method (Chr.8 Whole Chromosome Painting Probe, Cytocell) revealed the marker chromosome to be a derivative chromosome 8:

47,XX,+der(8)(pter→q21).ish der(8)(pter→q21)(wcp8+)[14]/46,XX[6]. The karyotypes of parents were normal.

In conclusion we identified a mosaic partial trisomy of chromosome 8 in a dysmorphic newborn. Discussion of possible etiology and clinical effect of this cytogenetical result will be presented.

#### P02.130

#### *De novo* cryptic deletion of 2q37 in a child with hypotonia and congenital heart defect

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Unbalanced cryptic chromosomal rearrangement involving the telomeric region is one of the major causes of complex genetic diseases resulting in mild to severe clinical conditions. We present here genetic studies on a one month old female child with hypotonia, poor feeding and congenital heart defect who was referred for chromosomal studies. Cytogenetic and FISH studies showed a *de novo* and unbalanced cryptic chromosomal rearrangement resulting from a transloca-

tion between chromosomes 2q37.1 and 20p13. The final karyotypic and FISH results were interpreted as 46,XX,der(2)t(2;20)(q37.1;p13).ish der(2)(2ptel+,2qtel-,20ptel+)dn, leading to trisomy of 20pter and monosomy of 2qter. Due to the severity of her condition she underwent cardiac surgery, but failed to survive. More than 60 cases of 2q37 terminal deletion have been reported so far with features ranging from developmental delay, mental retardation, dysmorphism, autism, cardiac or renal abnormalities etc. A subset of patients with this distal deletion, are reported to mimic Albright hereditary Osteodystrophy (AHO). Recent array CGH studies by Lukusa et al (2004) correlated 2q37.3 deletion with autism. Molecular cytogenetic studies using FISH and array CGH should be considered for patients presenting with hypotonia, feeding difficulties and failure to thrive. Literature review and genotype-phenotype correlation involving 2q37 microdeletion will be discussed.

#### P02.131

#### The facial dysmorphism in the newly recognised microdeletion 2p15-p16.1 refined to a 570 kb region in 2p15

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The implementation of new technologies such as array-based comparative genomic hybridisation (aCGH) in the genetic diagnosis screening of patients with mental retardation and multiple congenital anomalies (MR/MCA) enabled the detection of novel subtle chromosomal imbalances, as well as the refinement of already known chromosomal imbalances and, in some cases, the identification of the respective genes. Recently a novel 6.2 Mb microdeletion involving 2p15-p16.1 was reported in two patients with autistic disorder (AD) and MR/MCA with recognisable dysmorphic features.

While screening for genomic copy number variations with a 1 Mb resolution bacterial artificial chromosome (BAC) aCGH in patients referred for the aetiological diagnosis of MR/MCA, a *de novo* 2p microdeletion was detected and refined by fluorescence in-situ hybridisation (FISH) to a 570 kb region in 2p15 in a 16 year-old boy presenting with mild mental retardation and multiple congenital anomalies with facial dysmorphism, ecomorphic habitus, kyphoscoliosis and congenital heart defect. In a first step, Marfan and Williams-Beuren syndromes were excluded by, respectively, *FBN1* gene mutation screening and *ELN* gene locus specific FISH probe.

We compare our findings with those already reported and we discuss the phenotype-genotype correlations.

This report supports the evidence of a newly recognised microdeletion syndrome involving 2p15-16.1. We show that this smallest 570 kb deletion of 2p15 is most likely responsible for the characteristic facial dysmorphism in this syndrome.

#### P02.132

#### A case of interstitial (4)(31.23q34.2)*de novo* deletion, detected by GTG, FISH and m-CGH analyses in a boy referred by a programme of 22q deletion searching

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Terminal deletions of 4q are rarely described (less than 100 cases) and frequently misdiagnosed at first as "22q deletion suspicion". We present a 9 year old boy with global retardation, epilepsy, bilateral lips and cleft palate, unusual facial appearance, ASD II and some other anomalies. He was recruited to diagnosis by the 22q deletion searching programme, but karyotype analyses (lymphocytes, GTG 450 -800 bb., RBG, FISH) did not show any anomaly of 22<sup>nd</sup> chromosome, but surprisingly revealed deletion, described at first as 46,XY,del(4)(q34qter). Karyotypes of both parents were normal. More precise m-CGH analyses (2,44 OLIGO m-CGH Agilent) showed interstitial 25,6 Mbp deletion, mapped between loci 178055037 and 152423109 (39 Mbp higher to telomere). Final karyotype description was then corrected as follows: 46,XY,del(4)(q31.23q34.2). Possible correlations between ge-

netic map of deleted region and clinical features are now discussed. In all cases of del(22)(q11), suspected with negative results of typical cytogenetic analyses, possibility of subtle aberrations of 4q terminal region must be carefully considered.

#### P02.133

#### Interstitial deletion of the long arm of the chromosome 10: about a Tunisian case with del(10)(q23q25)

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We describe a Tunisian patient, a one month-old boy which is the second child of healthy unrelated parents. During pregnancy there was intra-uterine delay of growth.

The infant was premature and was born at 35 weeks of pregnancy. Birth weight was 1850 g, length was 49 cm, and head circumference was 33 cm.

There was a facial dysmorphism consisting of down slanting palpebral fissures, prominent forehead, broad nasal bridge, anteverted nares, thin lips, high palate, low-set ears, long philtrum and retrognathia. He had short neck, clinodactyly of the fifth fingers, a unique left kidney, shawl scrotum and club foot varus.

The cytogenetic analysis revealed deletion of the long arm of chromosome 10: 46,XY,del(10)(q23q25) in all mitosis. His parents showed normal karyotypes.

Interstitial deletions of 10q are rare, this report describes a boy with a de novo interstitial deletion of the long arm of chromosome 10. Typical presentation includes craniofacial dysmorphisms, postnatal growth retardation, digital anomalies, developmental delay, congenital heart defects and urogenital anomalies. The clinical findings are mainly the same as those reported in patients with interstitial deletion of this region especially facial dysmorphism, our patient has no congenital heart defect, why he has unique left kidney and most of the patients described have a deletion more distal than our deletion.

The breakpoint will be confirmed by FISH analysis and well permit to compare exactly the phenotype in this case with those described in literature were there is only few cases of this deletion.

#### P02.134

#### Deletion (1)(p32.2-p32.3) detected by Array-CGH in a Patient with Developmental Delay/Mental Retardation, Dysmorphic Features and Low Cholesterol: A New Microdeletion Syndrome?

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We report a 25-year-old male with developmental delay/mental retardation, low levels of total and LDL cholesterol and dysmorphism, which includes macrocephaly, hypertelorism, downslanting palpebral fissures, low set ears, bilateral cataracts, cleft palate, bilateral cleft lip and wide spaced nipples. While his karyotype and subtelomeric FISH studies were normal, a 5.4 Mb interstitial deletion at 1p32 [del(1)(p32.2-p32.3)] was identified by array-CGH. This region encompasses a cluster of genes involved in fatty acid oxidation and cholesterol metabolism. One of these genes is PCSK9 (proprotein convertase subtilisin/kexin type 9), which is a key regulator of the number of cell-surface LDL receptors. Another gene deleted is , DAB1 (Disabled 1 homolog of Drosophila), which is involved in brain development. Based on the findings in our patient and in the two previously reported individuals with del(1)(p32.2-p32.3), they may have a new microdeletion syndrome that previously has been difficult to detect by G-banding because it is located in a G-negative band, but it can easily be identified by array-CGH

#### P02.135

#### Identification of a 2.7 Mb deletion of 3q25.1-3q25.2 in a patient with complex rearrangements of chromosome 3 by oligo-array CGH

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Interstitial deletion of chromosome 3q23 is known in blepharophimosis-ptosis-epicanthus inversus syndrome (BPES). Recently, a 1.9 Mb deletion of 3q24 harboring ZIC1 and ZIC4 has been identified in Dandy-Walker malformation. We present a Korean boy with mild dysmorphism and congenital heart disease such as pulmonary atresia, VSD and major aortopulmonary collateral artery (MAPCA) at birth. He had a apparently balanced translocation, 46,XY,der(3)inv(3)(p25q25)t(3;8)(q29;q24.1),der(8)t(3;8) de novo, with complex rearrangements of chromosome 3. A high density array CGH with 244k oligonucleotide probes detected a 2.7 Mb deletion at 3q25.1-3q25.2. Further investigation by FISH analysis with BAC clones confirmed the heterozygous deletion at the same region. The genes COMMD2, RNF13, PFN2, SERP1, EIF2A, SELT, SIAH2, CLRN1, and several open reading frames are included in the deletion interval. Among them, some genes may be good candidates for the congenital heart disease or other phenotypes.

#### P02.136

#### A patient with a 6p interstitial deletion and a complex translocation involving chromosomes 2, 6, and 14

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We describe a 5 years old patient with global developmental delay. He lags about one year behind his peers. The language development is most delayed, especially the pronunciation. As a baby he was quiet and slept excessively. The patient has hypermetropia of 2.5 diopters bilaterally and minor exophoria. Eye examination revealed a choriretinal coloboma inferonasally in the left eye. He shows dysmorphic features: hypertelorism, deep set eyes, prominent filtrum, slightly prominent forehead, low set and backward rotated ears.

A combined approach of G banding, aCGH and FISH revealed complex chromosome rearrangements, involving chromosomes 2, 6 and 14. These rearrangements are *de novo*, since both parents have a normal karyotype. He also has two healthy sibs.

Beside the reciprocal translocation between chromosome 2q and 6p, we also detected an insertion of a large segment from chromosome 14 into chromosome 6p, and an extensively reshuffled 6p: chromosome der(6)p also carries a 4 Mb interstitial deletion and a small paracentric inversion.

Because of the high number of chromosome breakpoints in this patient we cannot connect the involvement of each breakpoint to the clinical phenotype. However, the most significant aberration is the 6p interstitial deletion. Interstitial deletions of 6p are rare events that to our knowledge previously have been reported in a total of eight patients. We compare the clinical traits of our patient to the few cases of 6p interstitial deletions previously described and we discuss the potential role of TFAF2A and FOXC1 in relation to the choroidal coloboma.

#### P02.137

#### Dicentric Inv Dup of the Whole 4p Without Deletion. Description of the First Case

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Terminal inversion duplications (inv dup) are relatively uncommon. The most frequent and well characterized inv dup, involved the 8p, where a maternal heterozygous inversion between the two OR gene-clusters, are causally related to the inv dup, and has always associated a terminal deletion and an intact segment 8p.

Here we describe a female patient with a dicentric inversion duplication 4p without any apparent euchromatin deletion.

The patient was a female newborn, first daughter of a young, healthy and non-consanguineous couple. Pregnancy was uneventful, until the 37th gestational week when a maternal hypertension was diagnosed. The delivery was induced at 38 weeks. At birth she showed microcephaly, a right cephalohematoma, craniofacial anomalies and skeletal anomalies of hands and feet.

High resolution G-band karyotype from peripheral blood lymphocytes, revealed a "de novo" 4p+ chromosome. FISH analyses with 4p probes, showed that the extra material on 4p was a dicentric inverted duplication (cen-p16.3::p16.3-qter).

We postulate that a chromatic breakage could have happened at the very terminal end of the chromosome 4, losing the common telomeric region (-TTAAGGG-) but saving the subtelomeric specific region. This was followed by U-type reunion producing a dicentric chromosome, which after a break at a centromeric region, gave rise to the abnormal dic inv dup 4p chromosome. The abnormal 4p was afterwards stabilized by the addition of a new -TTAAGGG- repeat sequence mediated by the telomerase, but surprisingly this sequence was internal to the centromere sequence.

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#### P02.138

#### DNA methylation patterns of extra chromosomes in chorionic villi cells of missed abortions

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Distribution of 5-methylcytosine (5-MeC) in human chromosomes reveals specific MeC-banding pattern. It remains unknown whether methylation pattern of extra chromosomes is the same as in normal euploid cells or it bears changes of functional significance. We compared DNA methylation patterns of extra chromosomes in cytotrophoblastic cells of missed abortuses with abnormal and euploid karyotypes. Methylation patterns of extra chromosomes in trisomies 9,7,13,16,17(one chorion sample for each case), in triploidy(three samples) and tetraploidy(one sample) as well as structurally rearranged chromosomes were studied on direct metaphase preparations from cytotrophoblast of human missed abortuses at 5-8 weeks of gestation.

Indirect immunofluorescence with monoclonal antibodies (Eurogentec,Belgium) was applied to detect chromosome regions, enriched in 5-MeC. No difference of 5-MeC signal distribution along chromosomes and its intensity between normal and aneuploid/polyploid cells was detected. In either case, 5-MeC-rich sites corresponded to T-,R-bands, short arms of acrocentrics and heterochromatin of chromosomes 1,9,16. Methylation intensity in homologues differed only in 9q12 and 16q11 (heterochromatin) in triads and tetrads.

DNA methylation pattern in structurally rearranged chromosome 7 (47,XX,i(7)(q10),+i(7)(p10)) was studied. The pattern 5-MeC signal distribution in both der(7) was band-specific and did not differ from that of the structurally normal one, as well as from homologues in normal karyotype.

Thus, methylation pattern in extra copies of normal and rearranged chromosomes is identical to that in normal karyotypes. Different methylation of 9q12 and 16q11 in homologues is more probably due to special role of heterochromatin in cytotrophoblast cells rather than change of methylation in aberrant karyotypes.

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#### P02.139

#### Unusual Clinical Manifestations Associated with Down Syndrome

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Down syndrome(DS) is by far the most common and best known chromosomal disorder.The cause is full trisomy 21 in the majority patients (94%). Mosaicism (2.4%) and translocations (3.3%) account for the rest. Most unbalanced translocations are de novo (75%), and the rest result from familial translocation. Here we report 3 cases of Down Syndrome presented with unusual clinical findings.

CASE 1: 3-month-old infant with DS was cytogenetically diagnosed as translocation type "der(13;21)" resulting from a Robertsonian translocation of the mother. Radiological evaluation confirmed an asymptomatic Morgagni Hernia.

CASE 2: 18-month-old DS patient presented with cleft lip-palate. Karyotype revealed regular Trisomy 21.

CASE 3: Cytogenetical analysis of a 2-month-old infant presenting DS showed 47,XY,+21 karyotype. Physical examination revealed a female external genitalia and inguinaly located bilateral gonads. Ultrasound confirmed absence of uterus.

Association of DS with cleft lip/palate, Morgagni hernia and androgen insensitivity is been rarely discussed. To our knowledge 32 cases have been reported so far; and for androgen insensitivity association only 3 cases have been referred. As up to date no androgen receptor gene mutation has been identified in similar cases, it is yet not clear whether this association is directly correlated. Cleft lip-palate is as well rarely reported in DS. Coincidental occurence could thus be discussed.

#### P02.140

#### Cytogenetic and parental age study of 545 cases of Down syndrome in Iran.A forty years study

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Objective: To determine the karyotypic and maternal age profile of Down syndrome in Iran this study started in 1965 and ended in 2004. Methods: 931 clinically diagnosed Down patients referred to the first author were studied. Peripheral bloods were cultured using Leukocyte culture method of Moorhead (1) or micro culture technique of Shariati( 2).Giemsa stained mitoses were analysed.Since 1975 G-banded mitoses at the 300-450 band resolution were karyotyped

Results: Karyotypes consistent with Down syndrome were observed in 545 patients out of 763 case.305 patients were male( 56 per cent ) and 240 cases were female(44 per cent ).66 per cent were born in Tehran while the rest were born across the country .Frequency of free trisomy 21,translocation 21 and mosaics were 89.5,5.3 and 5.2 per cent respectively.the mean age of parents was 34.67 (SD 9.14) years for Fathers and 28.49 (SD 7.71 ) for Mothers. 52 per cent of our cases were the result of first or second pregnancies..Only 11.3 per cent had consanguineous parents.

Conclusion: Our study of 545 cytogenetically proven Down syndrome patients show a rather different picture regarding age of parents and parity in mothers as compared to the western reports.In our study the mean maternal age is 28.4 with peak at 22 while in western countries it is 34.Also 52 per cent of our cases are the results of first or second pregnancies while 48 per cent are the results of 3rd to 18th pregnancies

#### P02.141

#### Pure 20q11.2 duplication: a specific behavioural phenotype?

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Pure 20q11.2 duplication has only been reported once to the best of our knowledge. We report a second case of pure 20q11.2 duplication in a 9 year-old-girl presenting mainly peculiar behaviour, a few dysmorphic features and no malformations. The proposita was born to non-consanguineous parents with uneventful histories. She was eutro-

phic at delivery. Minor neonatal problems included polycythaemia and neonatal jaundice. During the first months the baby was hypotonic. Physiotherapy achieved walking at 16 months. Course was marked by delayed speech with phonatory difficulties. Academic delay was due mainly to hyperactivity and impaired concentration. At 9 years intelligence was borderline (IQ:74). Dysmorphic traits included small dysplastic ears and a large mouth. There was strikingly happy and jovial behaviour. Karyotype analysis of RHG and GTG-banded blood metaphases showed 46 chromosomes, with an abnormally elongated long arm of one chromosome 20. Initial FISH studies (wcp 20, subtelomeric 20p and 20q probes and CEP 20 probe) suggested interstitial chromosome 20 duplication. Thorough FISH characterization of the anomaly concluded to partial trisomy 20q11.2 resulting from an inverted duplicated chromosome 20. A similar but apparently slightly larger duplication was reported by Wanderley et al. in 2005 in a 16-month-old boy with slightly dysmorphic features and the same happy disposition. This behavioural characteristic could be related to 20q11.2 duplication.

#### P02.142

#### **De novo duplication of 7(q21.2----q32) in a patient: cytogenetic diagnosis and clinical finding**

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Trisomy/duplication of 7q is associated with a characteristic syndrome and has been described in published cases. The main clinical features are: frontal bossing, retrognathia, small jaw, low-set ears, dysplastic ears, deep-set eyes, prominent eyes, strabismus, downcurved upper lip, small mouth, short hands, stiffness fingers, joint laxity, joint stiffness, scoliosis, reduced muscle tone, hydrocephalus, growth retardation, strabismus, coloboma of iris, drooping upper eyelid, widely spaced eyes, long eyelashes, short space between eyelids (1-8). Here we describe clinical and cytogenetic findings on a 1 year old male child whom referred to our clinic due to developmental delay and hypotonia.

To our best knowledge this report is the first case of a *de novo* case with pure partial duplication of 7 (q21.2---q32).

#### P02.143

#### **Rare unbalanced aberration of chromosome 18 in patient with severe dysmorphic features and poor prognosis**

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The unbalanced aberration of chromosome 18 is very rare chromosomal rearrangements. We present a patient with rare unbalanced aberration of chromosome 18. He was a first child of the first pregnancy from non-consanguineous parents. His mother was consulted during pregnancy at the Center for Medical Genetics. Risk of congenital malformations for foetus was calculated due to maternal age, gestation and obstetric history. The risk of trisomy 21 was 1:1099 according to the mother's age. Ultrasound scan was performed at 16<sup>th</sup> week of gestation. Neither foetal structural malformations nor minor defects or markers of chromosomal diseases were detected. Triple test was performed at 16<sup>th</sup> week of gestation. Biochemical risk of trisomy 21 was 1:55, for Trisomy 18 - 1:1708, for neural tube defects - 1:356. According to biochemical test results of trisomy 21 invasive procedure was performed for aneuploidy testing by QF-PCR. Test results were negative. At birth the weight was 2470g and dysmorphic features were characterized - microcephaly, low hairline, hypertelorism, prominent nasal bridge, long philtrum, short neck, overlapping position of the fingers, micropenis and corpus callosum agenesis. Expressed respiratory insufficiency was also observed. The death at sixth months was final outcome of this patient. Chromosomal analysis of peripheral blood lymphocytes revealed 46,XY,der(18)t(4;18)(p14;q12.2) karyotype. Cytogenetic analysis was performed from GTG banded metaphases. The resolution level was 400-500 bands. Cytogenetic investigation of the parents showed a chromosome aberration in mother: she presented a t(4;18) (p14;q12.2). These results identified the exact nature of the unbalanced aberration of our patient.

#### P02.144

#### **A syndrome of ectodermal dysplasia and MR due to del(2)(q31.2q33.2): further clinical and CGH-array characterization**

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We report a patient with a 23 Mb deletion at 2q23, presenting with severe growth and mental delay, and partial ectodermal dysplasia (hair and teeth involvement). This recurrent deletion appears to show a consistent phenotype, previously reported in 4 cases. Further patients will be necessary to determine which gene in the interval explain skin and developmental anomalies. Comparing clinical features from our patient and others who show an overlapping deletion, a common "del 2q32 phenotype" emerge: *microcephaly, long face, high forehead, abnormal teeth, low-set ears, midface hypoplasia, high palate, micrognathia, transparent and thin skin, sparse hair, high frequency of inguinal hernia, severe development impairment, and behavioral problems*. Anomalies of hands and feet are also common: this might be caused by the deletion of the HOXD cluster which is involved in distal limb morphogenesis. Cleft palate is due to the deletion of the SATB2 gene most likely by haploinsufficiency. Both ZNF533 and MYO1B genes are located in the deleted region. They are involved in neuronal function. These genes may be good candidates for the neurological phenotype which is however not present to date in our patient. Is there a new locus for ectodermal dysplasia on chromosome 2q31q33? This hypothesis is supported by the observations of Stenvik et al. (1972) who described patients with congenitally missing teeth and sparse hair and taurodontia. Nails and ability to perspire were not specifically mentioned. Levin (1985) saw brother and sister with hypodontia and sparse, slow-growing hair. The sister had taurodontia of deciduous and permanent teeth. In addition, fingernails and toenails were slow-growing, thin, and spoon-shaped. No abnormalities in perspiration were noted. (taurodontia, absent teeth, and sparse hair. MIM 272980)

#### P02.145

#### **A 6qter deletion results in a phenotype of mental retardation and classical cutaneo-articular Ehlers-Danlos syndrome**

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Ehlers-Danlos syndromes (EDS), belonging to a heterogeneous group of inheritable connective tissue disorders, are attributed to mutations within a subunit of the V<sup>th</sup> chain of the collagen gene. Here, we report on a 6-year-old patient with history of congenital hip dislocation, major joint hypermobility, fragile and hyperextensible skin, persistent atrophic scars, and asthenia. Her father and one of her sisters had mild joint laxity. She was referred to the genetics department for investigations of speech delay, mild mental and growth retardation and minor dysmorphic features. A standard karyotype revealed a 6qter deletion. Cytogenetic studies of the parents were normal in favour of a *de novo* deletion. A CGH-array (Integragen) is in progress to better characterize the breakpoints of this deletion. Cerebral magnetic resonance imaging, cardiac and abdominal ultrasounds were normal. It is interesting to note that two patients with EDS type VII and EDS type II respectively have been reported with a 6q deletion. None of the genes of this subtelomeric 6qter region seems to be a strong candidate gene by its function for EDS. The review of other cases with chromosome 6q deletions including the q26-q27 bands did not show any other cases with EDS phenotype but revealed patients with congenital hip dysplasia.

#### P02.146

#### **Encountered chromosomal abnormalities in children with epilepsy**

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Objective: Increased evidences of chromosomal aberrations in association with epilepsy suggest a genetic predisposition to the disease. We

aimed at identifying the frequency of chromosomal aberrations among children with epilepsy. Methods: Twenty Egyptian children with epilepsy were recruited for this study. Full history, clinical & neurological examination together with some investigations (EEG, MRI & CAT) was done. Chromosomal analysis using GTG banding and high resolution techniques were evaluated. FISH technique was done to one case only. Results: Chromosomal aberrations were observed in 3/20 (15%) of which one case had mosaic interstitial deletion 15(q11q13), the second case had mosaic inversion 22(q11q13), While the third case had a ring of chromosome 18. Conclusions : The possibility of chromosomal abnormality in cases of epilepsy without apparent etiology and / or associated congenital malformation or without frank dysmorphic features should be seriously acknowledged. Genetic evaluation including high resolution chromosomal study, FISH technique and molecular study should be used for proper management and counseling.

#### P02.147

#### Induced chromosomal breakage rate in children referred for aplastic anemia

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**Background:** Fanconi Anemia (FA) is a rare autosomal instability syndrome characterized by bone marrow failure, developmental anomalies, acute nonlymphocytic leukaemia and cellular hypersensitivity to cross linking agents such as diepoxybutane (DEB) and mitomycin C (MMC). However, a number of patients display only minor phenotypic variations or lack congenital anomalies.

**Material & Methods:** Chromosomal breakage analysis using MMC and DEB was performed to differentiate FA from aplastic anemia in 166 children aged from 2 months to 14 years with myelodysplasia with or without congenital malformations. Matched for age and sex donors were used as controls. Peripheral blood samples were analysed with conventional cytogenetic techniques. For clastogen-induced chromosome damage both MMC and DEB were added. A minimum of 150 metaphases per case were analyzed. FA positive was considered the case in which the percentage of breaks was 7-10 times higher as compared to controls.

**Results:** Induced breaks were detected in 8/166 patients tested with both clastogens. Six were diagnosed as FA while the remaining two, despite the high percentage of clastogenic damage, were characterised as Silver Russell syndrome and Blackfan Diamond anemia respectively. 3/6 FA patients presented with congenital anomalies and 3 only with aplastic anemia. 2/6 FA patients were dizygote twins. No relationship was found between the clinical severity of the disease, age of onset, and the anemic status.

**Conclusions:** The present study illustrates that clastogens induced stress tests provide the means of differentiation between FA and «aplastic anemia» and allow for accurate and timely diagnosis to implement appropriate therapy.

#### P02.148

#### Molecular cytogenetic characterization of the same translocation in two siblings

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We are reporting the clinical, molecular cytogenetic studies of two female siblings of 14 and 6 years, who were referred for karyotyping because of multiple congenital anomalies and mental retardation. The karyotypes of parents were normal. Phenotypes of proband were similar and included: spine deformity, mild microcephaly, large nose with high nasal bridge, micrognathia and crowded teeth. Conventional cytogenetic analysis of both siblings using GTG-banding revealed 46, XX, der(15) karyotype. FISH analysis with mFISH probe kit (MetaSystems), wcp15, wcp 8, telomere specific for chromosome 8 DNA-probes (Abbott) was performed to identify derivative chromosome. mFISH showed the origin of the material from the chromosomes 8 and 15. FISH with wcp15, wcp 8 confirmed this date and 8q telomere was seen on the derivative chromosome. Thus the derivative chromosome was composed from the part of long arm of chromosome 8 and whole chromosome 15. For detailed karyotype description DNA probes were generated

from abnormal chromosomes followed with DOP-PCR and labeling of PCR products in additional cycles of PCR. In patients FISH of these microdissected DNA probes painted abnormal chromosome 15(p11.2-qter) and 8(q22.1-qter). In healthy donors they painted 15(p11.2-qter) and 8(q22.1-qter). Technique of M-bands with DNA probes derived from two different mar(15) revealed no additional reorganization in pericentromeric region of abnormal chromosomes. Obtained data allowed us to describe abnormal karyotypes as 46, XX, der(15)t(8;15)(q22.1;p11.2). The normal karyotypes of both parents led us to hypothesis that this der(15) is probably a result of parental gonadal mosaicism. Different biological father cannot be excluded

#### P02.149

#### MCA/MR Syndrome with (4; 10) (q25; q26) Translocation

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We describe a 52 days boy with low birth weight, sparse scalp hair, absent eye brows and eye lashes and bilateral corneal opacities, umbilical hernia and anal stenosis. Limb anomalies in the form of bilateral syndactyly between first, second and third fingers, bilateral lower limb preaxial polydactyly and bilateral soft tissue syndactyly between second and third toes are described. The karyotype of the infant revealed a unique de novo translocation involving chromosomes 4 and 10, which was confirmed by Fluorescence In Situ Hybridization (FISH) technique, resulting in t(4;10)(q25;q26). No other patients, to our knowledge, with an identical phenotype and chromosomal finding have been reported. Our report suggests that regions 4q25 and 10q26 may be involved in the development of the limb anomalies, eye anomalies and other characteristic clinical findings presented in our patient.

#### P02.150

#### Detecting of gonadal mosaicism for trisomy of chromosomes without severe imprinting effects

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Gonadal mosaicism (GM) may account for both recurrent cases of chromosomal anomalies and appreciable proportion of "sporadic" cases. However even in the case of a recurrent anomaly, it is not always easy to discover a suspected GM in the absence of the abnormal line in a somatic tissue of a carrier. To optimize the testing procedure, we have conceived the following algorithm. (i) Study on parental origin of the extra chromosome in the trisomic offspring. (ii) Finding apparent non-disjunction (NDJ) is followed by testing the grandparents on the parent-of-origin's side. Presence of two homologs from the same grandparent in the parent-of-origin (uniparental disomy, UPD) and in the trisomic proband, would indicate GM in the parent-of-origin. (iii) Finding a "new" extra chromosome in the proband is followed by testing the grandparents on both sides. Presence of a grandparental homolog not seen in both parents in the proband, would uncover a carrier of GM. Presence of crossovers ("new" allele) may add to uncovering both GM and its carrier. Finding UPD or a "new" chromosome/allele in a healthy offspring may also help to reveal the presence of GM in a parent. (iv) Parents-of-origin with undetectable GM should receive a rigorous multi-tissue cytogenetic investigation for the presence of abnormal line. We believe that expenses of labour- and time-consuming testing of families for suspected GM can be rewarded by the opportunity of choosing a desirable reproduction strategy. Results of a pilot study of 30 families with trisomy 21 offspring will be presented.

#### P02.151

#### Karyotyping of early passaged human mesenchymal stem cells

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Human mesenchymal stem cells (hMSC) have a great potential for a wide range of therapeutic purposes. Their medical application necessarily requires preliminary passaging. The question of unfavorable effects of passaging of hMSC on their genome (chromosome) stability remains controversial.

We have studied genome stability of hMSC, obtained from 6 healthy

(according to questionnaire) volunteer donors by bone marrow aspiration. To test genome stability, we performed karyotyping by QFH-banding technique with AcD-counterstaining.

First, we made a comparative analysis of hMSC karyotype with that of relevant dcPHA-stimulated lymphocytes, cultured under standard conditions. Lymphocytes demonstrated normal male or female karyotypes in either donor. Comparative analysis of metaphase chromosomes showed perfect karyotype concordance in both hMSC and lymphocytes.

Second, we analyzed hMSC karyotype at early passages - from 4 to 7. Metaphase chromosomes were obtained by colchicines treatment 5 hours before fixation; 8-15 metaphases were selected for karyotyping in each case. No differences in hMSC karyotype among cells of the same culture as well as among cells from cultures after different passages could be detected. No aneuploid or polyploid cells as well as no cells with structural chromosome rearrangements were registered.

Thus, no adverse effect of cell passaging on chromosome number or their morphology could be detected, advocating for genome stability of hMSC at early passages.

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#### P02.152

##### Familial chromosome 9 balanced intrachromosomal insertion leading to offspring with a 9Q34

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Shifts or intrachromosomal insertions represent very rare complex three-break rearrangements. They are classified in peri- or paracentric insertions depending on their occurrence within one or two arms. They can be direct or inverted depending on the orientation of the inserted segment with regard to the centromere. The normal carriers have a high reproductive risk.

We report on a family with a chromosome 9q paracentric insertion discovered through a newborn male referred for cytogenetic analysis because of the association of facial dysmorphism, radio-ulnar synostosis, undescended testes and hydronephrosis. A recombinant chromosome 9 was found. Parental karyotypes showed a paternal intrachromosomal insertion of a part of 9q34 band. By using molecular cytogenetic techniques (CGH-array and FISH), we characterized the break points of the insertion. After chromosomal study of the family, four other carriers of the insertion were found. A prenatal diagnosis could be proposed to a pregnant woman whose husband was a normal carrier and a 56 year old woman with an unexplained abnormal phenotype was identified as a carrier of the recombinant chromosome 9. We discuss the mechanism which have led to this duplication.

#### P02.153

##### Paternal origin of a large inv dup(15) supernumerary marker: no abnormal phenotype at 2 years old

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Inv dup(15) can be classified into 2 major groups according to size, determined by presence or absence of Prader -Willi/Angelman syndrome critical region (PWACR). Small inv dup (15), not containing PWACR seems to have no phenotypic effect whereas large ones, containing 2 or more extra copies of the 15q11q13 region, are associated with abnormal phenotype. In those cases, inv dup(15) are maternal in origin. We report on a prenatal observation with a mosaic marker confirmed after birth. The proband is the second child of a young, healthy and unrelated parents. Prenatal diagnosis performed due to increased fetal nuchal translucency at 22 weeks of gestation indicated cytogenetic mosaicism for a supernumerary marker. The low level of mosaicism did not permit to identify it: 47,XX,+mar[3]/46,XX[52]. Normal karyotypes were found in both parents.

After birth, the karyotype was analysed on different tissues and all confirmed mosaicism for the marker: 45% for blood sample and 25% for fibroblasts. FISH studies with commercial probes and BACs probes from 15q11q13 region allowed us to characterize the marker as an inv dup (15)(q11q12) including the Prader-willi/angelman critical region. Moreover, molecular studies for parental origin demonstrated a paternally derived inv dup (15).

At 2 years old the young girl has a normal phenotype with no dysmorphic features and no psychomotor retardation.

Conclusion: we report on a young child with a large mosaic inv dup(15) whose paternal origin was rarely reported and supposed to be the explanation for the good psychomotor development of this child.

#### P02.154

##### The recurrence of pericentric inversions of chromosome 12 in the Tunisian population

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We report a recurrent pericentric inversion of chromosome 12 in five Tunisian families. It was ascertained through cytogenetic analysis of 3 men investigated before ICSI treatment for idiopathic infertility and two sisters explored because of IVF attempts failure and recurrent early pregnancy losses. The fifth family was ascertained through a prenatal diagnosis because of a familial history of a chromosome 12 inversion in an infertile female member. In these families, there was no familial occurrence of patients with mental retardation or multiple congenital anomaly syndromes, suggesting that unbalanced recombinations seem to be lethal. The same inv(12) was reported by other Tunisian cytogenetic laboratories, in association with infertility or recurrent abortions.

These pericentric chromosome 12 inversions have two different breakpoints: inv(12)(p11q12) shown in three families originated from Sfax town and inv(12)(p12q11) revealed in two families originated from suburbs of Sfax town.

This inversion may have an independent occurrence from different ancestors or has been transmitted from a common founder. Furthermore, observation of 2 different breakpoints can be explained by genomic structure of chromosome 12 which contains numerous duplicated and repetitive sequence elements that could mediate the formation of this recurrent inversion. We propose that pericentric inversion of chromosome 12 is a recurrent observation in Tunisian population and need characterization of breakpoints at the molecular level, to ascertain whether the formation of the inversion is mediated by repetitive sequence elements and haplotype analysis, to determine the proportion of inv(12)s that arose independently and the proportion that share an ancestral founder.

#### P02.155

##### Unexpected prenatal detection of recombinant chromosomes derived from pericentric inversions: report of two cases

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Pericentric inversions, excluding variant forms, are rare chromosomal abnormalities and have an estimated frequency ranging from 0.12% to 0.7%. During meiosis, recombination could lead to the formation of unbalanced recombinant (rec) chromosomes. Two alternative recs are possible: duplication of q-arm and deletion of p-arm or duplication of p-arm and deletion of q-arm. The probability of recombination increases with the size of the inverted segment. However, the viability of the recombinants depends on the length of the non-inverted segments. We report two prenatal diagnoses of fetuses with recombinant chromosomes resulting from parental inversions recently detected in our laboratory. Case 1: CVS was performed on a 30-year-old pregnant due to edema, increased nuchal translucency and suspicious of cardiopathy. The karyotype showed a derivative chromosome 14 with partial deletion of q-arm. Subtelomeric MLPA showed 14qter deletion. Cytogenetic analysis of the mother showed that she was carrier of an inv(14)(p11.1q24). The pregnancy was terminated and the pathological examination confirmed tetralogy of Fallot. The karyotype of the fetus was described as 46,XY,rec(14)dup(14p)inv(14)(p11.1q24)mat. Case 2: CVS was performed on a 32-year-old pregnant due to increased nuchal translucency and positive screening for Edwards's syndrome (1/10). Cytogenetic analysis showed extra material on chromosome 4p. Subtelomeric MLPA showed 4pter deletion and 4qter duplication. After karyotyping the parents, the father proved to be carrier of an inv(4)(p15.3-p16q33), so the karyotype of the fetus was described as 46,XX,rec(4)dup(4q)inv(4)(p15.3-p16q33)pat. First trimester cytoge-

netic study allows the correct diagnosis of such cases and provides an early genetic counselling in patients with high recurrence risk.

#### P02.156

#### Inversions detected in a study of 79920 prenatal and postnatal karyotypes

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Inversions are intrachromosomal rearrangements apparently balanced, generally without consequences for the carrier; however, an abnormal phenotype may be expressed if a breakpoint disrupts a critical gene. The observed phenotype depends on the length of the inverted segment, number of chiasmas produced and the genes of the involved region.

The incidence of pericentric and paracentric inversions in general population ranges from 0,12 to 0,7/1000 and 0,1 to 0,5/1000 in newborns respectively. The frequency in the general population is estimated at 1-2%. It is 13 times higher among infertile men than in general population. Indeed, these chromosomal abnormalities can perturb spermatogenesis and lead to the production of unbalanced gametes.

Out of 79920 cases that came in for genetic counseling during the last 6 years, we have detected 146 chromosome inversions, excluding polymorphic inversions of chromosome 9. Among these cases, 63 have been prenatally detected and 83 postnatally, 108 are pericentric inversions and 33 cases are inherited.

We have identified inversions for chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 18, 20, X and Y.

Abnormal phenotype has been observed in 5 postnatal cases (3,4%). In 8 cases, the reason for referral was infertility or miscarriages, nonetheless ascertainment was primarily incidental.

In conclusion, our study has revealed an incidence of inversions in agreement with what it was expected. According to the literature, we have found that the presence of an inversion is rarely linked with an abnormal phenotype or abnormal progeny, nevertheless caution is recommended when counseling.

#### P02.157

#### De novo isodicentric X chromosome: report of two new cases

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Isodicentric X chromosomes with an Xq deletion are uncommon. Phenotypes are variable and correlate with the existence of mosaic or non-mosaic isodicentric X chromosomes and also depend on the amount of deleted genetic material in the patients.

We report two new cases which were referred to our Department due to primary amenorrhea. First case was 20-year-old, 158 cm height, 54 kg weight and with intermediate IQ. She presented with the typical features of Turner syndrome: broad and shield-shaped thorax, small breasts, wide-spaced nipples, cubitus valgus, short and thickened neck, low hairline, scant axillary and pubic hair, infantile external genitalia. Sonography report showed lack of ovaries and hypoplasia of uterus. Her thyroid hormones were normal but her FSH and LH were high. Karyotyping was performed on peripheral blood lymphocytes using different banding techniques according to standard methods. Her karyotype was: 46, X, idic (X)(q24). Chromosome studies of her parents and her three sibs revealed normal.

Second case was 18-year-old, 155 cm height, 62 kg weight and sub-normal intelligence. Her clinical features were: shield-like chest, no breast development, widely spaced nipples, pigmented nevi, normal neck, absence of axillary hair, limited pubic hair, infantile external genitalia. Her sonography revealed horseshoe kidney, hypoplastic uterus and absence of gonads. She had elevated FSH and LH. Her karyotype was: 45, X [80%] / 46, X, idic (X)(q22) [20%]. Cytogenetic investigations of her parents and her four sibs revealed normal.

#### P02.158

#### Jumping translocation in a phenotypically normal male - a study of mosaicism in spermatozoa, lymphocytes and fibroblasts

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Jumping translocation refers to a rare type of mosaicism, in which the same chromosome segment is translocated to different chromosome sites in different cell lines. We report a case of mosaicism for two cell lines, each cell line containing a different de novo Robertsonian translocation with the common breakpoint in the centromeric region on chromosome 13, in a phenotypically normal male. The karyotype was defined as: 45,XY,der(13;13)(q10;q10)/45,XY,der(13;15)(q10;q10)de novo. The relative occurrence of the two clones in lymphocytes, fibroblasts and spermatozoa was determined using karyotype analysis and fluorescence in situ hybridisation (FISH). Karyotype analysis of lymphocytes revealed 57 % der(13;13) cells and 43 % der(13;15) cells and for skin fibroblasts the figures were almost identical (56 % and 44 % respectively). FISH analysis showed 55 % balanced nuclei for unselected spermatozoa and after swim-up selection the number of balanced spermatozoa decreased to 41 %. In addition, 16 % of the unselected spermatozoa and 27 % of the spermatozoa after swim-up selection carried an additional chromosome 13, indicating a high risk for trisomy 13 offspring (Table 1). To the best of our knowledge this is the first study on meiotic chromosome segregation in spermatozoa from a jumping translocation carrier as well as a Robertsonian der(13;13) carrier. The reproductive significance of the abnormality and its implications will be discussed.

**Table 1** Results from FISH-analysis of one thousand spermatozoa

Chromosome	13/15	13/15*	13/15*	13/15	13/15	13/15	13/15	13/15	13/15
No of signals from chromosome 13/15	1/1	0/1	2/1	1/0	1/2	2/2	2/0	3/1	
% of spermatozoa	Before swim-up selection	54,80	26,10	15,70	1,80	0,80	0,20	0,30	0,30
	After swim-up selection	40,60	30,50	26,60	1,50	-	-	0,30	0,50
Segregation mode	Alternate	Adjacent				3:0†	Other††		

\*These columns also contain segregation products of the homologous Robertsonian translocation der(13;13), which are not true adjacent segregation. †Using this two-colour FISH approach, it is not possible to differentiate between diploid spermatozoa and 3:0 segregation as both show two hybridisation signals for the probes used. †Nuclei with an unexpected combination of signals according to the possible segregation modes are classified as other.

#### P02.159

#### Low level mosaicism homologous Robertsonian translocation (21;21) in a mother of boy with unbalanced der(21;21)

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Parents of fetuses and children with unbalanced homologous acrocentric rearrangements are rarely found to be carriers or mosaic for the same rearrangements. Carriers of homologous acrocentric rearrangements (Robertsonian translocation) phenotypically normal they are at very high risk of having multiple spontaneous abortions and chromosomally abnormal offspring. Very frequently fetuses with homologous Robertsonian translocation arise postzygotic and are of maternal and parental origine.

We report about a fenotypically healthy woman with low level mosaicism for Robertsonian translocation (21;21). She delivered her first child in 21 year and he had Down's syndrome. Chromosomal preparations were made and banded using standard techniques. The karyotype of the boy was 46,XY,t(21;21),+21. After genetic counseling were made analysis parents. Father had normal karyotype 46,XY. Mother's chromosomes analysis showed very low level mosaicism, karyotype 46,XX [95]/45,XX,t(21;21) [5], which was detected because analysis made in a large number of cells.

After prenatal chromosomal diagnostic this woman delivered two healthy boys. Fourth pregnancy was aborted because prenatal cytogenetic analysis fetus showed unbalanced Robertsonian translocation. Identification of mosaicism allows for accurate genetic counseling and discussion of reproductive options.

**P02.160****Characterization of nine small supernumerary marker chromosomes detected in 7,000 fetal karyotypes**

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<sup>1</sup>Genomedica, Athens, Greece, <sup>2</sup>Bioiatriki S.A., Athens, Greece, <sup>3</sup>Embryo Research Center, Athens, Greece, <sup>4</sup>Research Center for Prenatal Diagnosis, Larissa, Greece, <sup>5</sup>Institute Of Human Genetics and Anthropology, Jena, Germany. Small supernumerary marker chromosomes (ssMCs) are structurally abnormal chromosomes that cannot be characterized unambiguously by conventional cytogenetic banding techniques. Until recently, the great variety of marker chromosomes and difficulties with their identification presented a problem for the cytogenetic interpretation of the karyotype. The risk for an abnormal phenotype is about 7% when de novo ssMCs derived from chromosomes 13, 14, 21, and 22 are ascertained prenatally.

In a Greek survey of 7,000 amniotic fluid samples, nine fetuses with marker chromosomes were detected in our laboratory. Using combined approaches of conventional cytogenetics including special staining techniques and fluorescence in situ hybridization (FISH), we successfully characterized all of them, which assisted subsequent genetic counseling and decision making. Eight of the SMCs were proven to be of autosomal origin. Of the autosomal SMCs, two originated from chromosome 15, one from chromosome 9, one from chromosome 18, one from chromosome 20, and three from chromosome 22. One marker chromosome was of sex chromosome origin. Euchromatin material was detected in 7 cases. Five of seven de novo marker chromosomes were associated with abnormal findings and were terminated. Our study demonstrates that molecular cytogenetic characterization of marker chromosomes detected at prenatal diagnosis, is of crucial significance to risk evaluation and decision making of the couple.

**P02.161****A complex chromosome 7q rearrangement identified in a patient with mental retardation, anxiety disorder and autistic features**

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We present a 15 year old girl, the second child of healthy parents. The girl is mentally retarded, suffers from a severe anxiety disorder and has autistic features. Her mean IQ was 48. She has a coordination disorder and hypotonia. She has small palpebral fissures, simply formed low set ears, abnormal palmar creases, scoliosis, long halluxes and her thumbs are proximally implanted. Length and skull circumference were -1 and -2.5 SD respectively. G-banding indicated a *de novo* paracentric inversion 46,XX,inv(7)(q31.3q34). SNP-array analysis, however, revealed a ~10Mb, 7q21.11-7q21.3 deletion in the paternal chromosome. Subsequent FISH analysis with ± 90 BAC/PAC clones in the 7q21-q35 region confirmed this deletion, but the expected paracentric inversion turned out to be an intra-chromosomal insertion of the fragment 7q31.31-q35 into band 7q21.3. PAC clone RP4-545C24 spans the breakpoint in band 7q35, positioning the breakpoint between the genes *NOBOX* and *TPK1*. The 7q31.31 breakpoint disrupts clone RP5-1047E14 and the predicted gene *NP079189*.

Six other patients with a deletion of 7q21.1-q21.3 have been reported previously. They share with our patient the following clinical features: mental retardation/developmental delay, microcephaly, mild facial dysmorphism, hypotonia, ear anomalies and small palpebral fissures. Our patient did not show any hearing loss, genital anomalies or growth deficiency, found in all of the previously reported patients. However none of the previous cases were reported to have anxiety disorder and/or autistic features. Therefore it is possible that disruption of the *NP079189* gene contributes to the anxiety disorder and the autistic features in our patient.

**P02.162****Cytogenetics Results in 301 Consanguineous Iranian Patients with Mental Retardation**

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Mental Retardation (MR) has heterogeneous etiology mostly with genetic causes. Chromosomal aberrations are one of the most common causes of MR and are responsible for 4-28% of all the mental retardation. In order to identify genes involved in mental retardation, 301 Iranian families with consanguineous marriage and positive family history for MR have been investigated in Genetics Research Center in collaboration with Max Planck Institute in Berlin. In these families at least two sibs were affected. As a first step, both chromosome investigation and fragile-X screening were carried out to exclude any abnormality. Molecular investigations were done on all the normal karyotype and fragile X negative patients. Standard Cytogenetics techniques using high resolution and GTG banding were carried out on all the patients. The overall chromosome abnormality rate was 3% (8 patients). The abnormalities included der(18)t(4;18)(q31.1;q23)mat, der(22)t(21;22)(q11;q13), 46,XY karyotype with female phenotype, and premature chromosome condensation in three different MR patients with microcephaly. However, our abnormality rate is lower than the reported cases. This is due to biased referral reasons, as all our cases are due to consanguineous marriage and therefore suggestive of autosomal recessive inheritance.

**P02.163****Premature chromosome condensation, Microcephaly and Mental Retardation: A report of three large consanguineous Iranian families**

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We report three out of a total of 50 Iranian consanguineous families affected with microcephaly, growth retardation, and mental retardation. These three families were unrelated and from northern and southern parts of Iran. Six members of one family and two of the other two were affected. Parents in all families were first cousins. Chromosome analysis using GTG banding technique on the probands showed a high frequency of prophase-like cells (80%) compared to normal controls (13%). The morphology of chromosomes was very poor and appeared fragile, twisted, curly and with raised breakage and overall very unusual looking. This phenomenon is due to premature chromosome condensation. Molecular investigation in the first family, using array-based homozygosity mapping and array CGH revealed deletion in the MCPH1 gene, one of four genes that have previously been implicated in autosomal recessive mental retardation with microcephaly. In the other two families, using homozygosity mapping, linkage to MCPH1 locus was suggested. MCPH1 gene is the only microcephaly gene associated with premature chromosome condensation.

The Cytogenetics findings demonstrating premature entry of cells into mitosis suggested mutation of genes involved in cell cycle regulation, which was then confirmed by molecular investigation. Our findings emphasize the importance of chromosome studies on patients with mental retardation and microcephaly. Findings of chromosomes with premature condensation in such patients implicate mutation in MCPH1 gene, which would therefore warrant further molecular investigations.

**P02.164****Pseudo-Angelman phenotype in two patients with a 2q23.1 microdeletion identified by array-CGH**

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**Background:** Genome-wide screening of patients with mental retardation using Array Comparative Genomic Hybridization (array-CGH) has identified several novel imbalances. With this genotype-first approach, the 2q22.3q23.3 deletion was recently described as a novel microdeletion syndrome. We report two unrelated patients with a *de novo* interstitial deletion mapping in this genomic region and presenting similar "pseudo-Angelman" phenotypes, including severe psychomotor retardation, speech impairment, epilepsy, microcephaly, ataxia and behavioural disabilities.

**Methods:** The microdeletions were identified by array-CGH using oligonucleotide and BAC-arrays, and further confirmed by Fluorescence *In Situ* Hybridization (FISH) and semi-quantitative PCR.

**Results:** The boundaries and sizes of the deletions in the two patients were different but an overlapping region of about 250 kb was defined, which mapped to 2q23.1 and included two genes: *MBD5* and *EPC2*. The *SIP1* gene associated with the Mowat Wilson syndrome was not included in the deleted genomic region.

**Discussion:** Haploinsufficiency of one of the deleted genes (*MBD5* or *EPC2*) could be responsible for the common clinical features observed in the 2q23.1 microdeletion syndrome and this hypothesis needs further investigation.

#### P02.165

#### Etiological Investigation of the Midline Facial Defects with Hypertelorism by Molecular and Cytogenetic Techniques

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The Midline Facial Defects with Hypertelorism (MFDH) are a heterogeneous and rare group of craniofacial disorders mainly characterized by ocular hypertelorism and bifid nose. The pathogenesis of these conditions is still unknown. All 14 individuals in this study were previously investigated by clinical, dysmorphologic and neurological evaluation, skull and facial X-rays, computerized tomography and MRI of the brain, and ophthalmologic and otorhinolaringologic evaluation and GTG banding. The evaluations demonstrate facial alterations, structural and functional anomalies of the central nervous system, indicating mainly, cortical migrations errors, perfusion variances and cerebella involvement. Based upon these observations, we determined an initial molecular investigation strategy. The phenotypes and a review of the literature suggested genes related to face and CNS development such as *SHH*, *PAX3* and *FGF8* may be involved in these disorders. These genes have been reported to participate in embryological development and are associated with some syndromes with craniofacial anomalies. The *SHH*, *PAX3* and *FGF8* genes were screened by direct sequencing and however mutations were found. To complement these studies, the whole genome tiling path array-CGH technique was performed and one deletion was found that affected *PAX3* in a familial case. Other copy number changes were detected and these findings are currently being confirmed by FISH and PCR. These preliminary results suggest our initial hypothesis that developmental genes, such as *PAX3*, play a role in the MFDH etiology.

#### P02.166

#### Diagnosis of Miller-Dieker syndrome (MDS) by fluorescence in situ hybridization (FISH)

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**Introduction:** Mieller Dieker Syndrome (MDS) involves a deletion of the chromosomal band 17p13.3 which contains the gene called LIS 1 (lissencephaly-1). It is a syndrome with a very low frequency, estimated in 11,7 cases for each million of births, although the incidence and the prevalence are probably higher.

**Objective:** The aim of this study is to report a case from "breast-fed baby" with an extensive record: premature baby, dysmorphic phenotype, lissencephaly, agenesis of the corpus callosum and septum pellucidum. Molecular techniques are used to confirm the submicroscopic

deletion on 17p13.3.

**Methods:** It was realized a double culture of lymphocytes with conventional banding GTG high resolution, followed by a FISH with the probe LSI 1 (maps to the 17p13.3 region on chromosome 17 containing the gene localization of the MDS).

**Results:** The fetus's karyotype is: Male: 46, XY, del (17) (p13.3). After hybridization with the probe, the SpectrumOrange LSI 1 signal was present in only one chromosome 17 and the SpectrumGreen LSI RARA 17q21.1 signal (control) was present in both chromosomes 17. Male: 46, XY, ish del (17)(p13.3 p13.3)(LIS1-).

The parents' karyotypes are in progress.

**Conclusions:** Our results postulate that approximately 90 percent of the patients with MDS phenotype show deletion of the band 17p13.3 but only in a 50 percent of these cases the deletion is visible by high resolution cytogenetic techniques. The own specificity of FISH achieves cytogenetic diagnosis that rarely it is obtained with banding techniques, unless prometaphasic chromosomes with high number of bands would be used.

#### P02.167

#### The results of missed abortus testing from Istanbul Memorial Hospital

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**INTRODUCTION:** The aim of this study is the retrospective data collection for patients underwent IVF treatment and spontaneous pregnancy lost between 2000-2007 for missed abortus with indications such as; advanced maternal age (ama), recurrent implantation failure (rif), recurrent pregnancy lost (rpl) and male factor.

**MAT- METHOD:** Tissue cultures were performed for missed abortus materials. Culture developments were observed and underwent harvesting steps in the optimum timing. Slides were stained with giemsa staining techniques (GTG) and 30 cells were counted.

Between years 2000-2007, tissue cultures were performed to 308 patients in our center. 149 of them were IVF patients, and 159 were spontaneous missed abort.

38/149 IVF and 48/159 spontaneous missed abort cases had abnormal karyotypes. We also analysed Trisomy 9, Trisomy 8, Trisomy 4 and Mosaic X in 4 PGD cases. The indications and chromosome analysis results of 38/149 IVF cases are represented in the table.1 below.

Table 1

Indications	Results
RPL (n=3)	Trisomy 8 (n=1) Triploidy (n=1) Trisomy X/ Trisomy 20 (n=1)
AMA (n=4)	Trisomy 13 (n=1) Trisomy 16 (n=1) Trisomy 18 (n=1) Trisomy 13/16 (n=1)
RIF (n=22)	Trisomy 3 (n=1) Trisomy 13/16 (n=1) Trisomy 12 (n=2) Trisomy 16/20 (n=1) Trisomy 13 (n=1) 45,X (n=1) Trisomy 15 (n=1) 47,XXY (n=1) Trisomy 16 (n=4) Tetraploidy (n=3) Trisomy 18 (n=1) del(1)(q32.1q42.1) (n=1) Trisomy 19 (n=1) der(14;14),+14 (n=1) Trisomy 20 (n=1) Trisomy 22 (n=1)
Male Factor(n=9)	45,X (n=4) Trisomy 16 (n=1) Trisomy 4 (n=1) inv(10q) (n=1) Trisomy 7 (n=1) Trisomy 9 (n=1)

#### Spontaneous missed abortus results (n=48)

Trisomy 3 (n=1)	Trisomy 21 (n=3)
Trisomy 6 (n=2)	Trisomy 22 (n=6)
Trisomy 7 (n=1)	Tripletoidy (n=5)
Trisomy 8 (n=3)	Tetraploidy (n=1)
Trisomy 16 (n=5)	45,X (n=11)
Trisomy 15 (n=5)	Mosaic X (n=1)
Trisomy 17 (n=1)	
Trisomy 18 (n=1)	
Trisomy 20 (n=2)	

**DISCUSSION:** In this study, we present that performing tissue cultures and karyotype analysis approve that PGD is a reliable option for the patients with several indications to have a non-affected offspring.

#### P02.168

#### Chromosomal Abnormalities in a Referred Population for Karyotype, Role of FISH to Refine the Diagnosis

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The aim of this study was to evaluate the cytogenetic findings in Egyptian cases referred for suspected chromosomal anomalies and to clarify the role of FISH to refine the clinical diagnosis. Cytogenetic study was performed on 4890 cases referred to Human Cytogenetic Department, National Research Centre. Referrals were grouped into 20 different categories of which genetic counseling represented the highest group 19.2% followed by short stature 12.4%, repeated abortions 10.2%, MCA/MR 10% Down syndrome 9.2%. Chromosomal aberrations were identified in 17.8% of cases, sex chromosome abnormalities represented 22% of the abnormal chromosomes. The most common autosomal abnormality was Down Syndrome 52%. In structural chromosome aberrations of autosome, deletion was the most common 6.8%, inversion 6% and translocation 5.2%. Different FISH probes were used when indicated. It was applied in 10% of referred cases as in cases of microdeletion syndromes, marker chromosomes, cryptic translocation, sex chromosomes identification and subtelomeric deletion. Accurate and refine cytogenetics and molecular cytogenetics diagnosis in our cases was the corner stone in proper genetic counseling.

#### P02.169

#### Characterization of rare karyotype anomaly 45,XX,-21/46,XX,r(21)by comprehensive FISH: mosaic status and constitution of ring chromosome 21

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Mosaic combination of monosomy 21 and partial monosomy due to ring chromosome 21 is a very rare finding in human karyotype. Here we describe a 2,5 years old female who has been referred to genetic department because of multiple congenital malformations (microphthalmia, cataract, corneal opacity, cerebellum abnormality, heart defect (ASD), craniofacial dysmorphias) and mental/growth retardation. Parents were young healthy non-consanguineous couple.

Conventional cytogenetic study of affected child revealed diagnosis 45,XX,-21[160]/46,XX,r(21)(p11q22)[160] de novo (parental karyotypes were normal). Somatic aberrations of ring chromosome in clone with r(21) were not found.

Molecular cytogenetic analysis was performed to characterize the constitution of r(21). Following probe sets were used for that: subtel 21q (Abbott/Vysis), BAC 105E1 (21q21.1), BAC 143N1 (21q21.3). Subtelomeric segment has been deleted by ring chromosome formation, and finally r(21) was described as ish r(21)(p12q22)(BAC 105E1+, BAC 143N1+, subtel 21q-).

Possible mechanisms of the mosaic karyotype abnormalities formation, clinical and cytogenetic aspects of examination of the patient, and data of the literature are discussed.

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#### P02.170

#### Multiple chromosome abnormalities in a case of neuroblastoma

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Chromosomal abnormalities play pivotal role in the process of malignant transformation of neural cells which is named as neuroblastoma. This malignancy has been studied extensively by various techniques like cytogenetics, flowcytometry, molecular methods, histopathology etc. to identify the genetic markers for their classification and gather information on significant prognostication for selection of appropriate choice of treatment. The most common genetic events associated with neuroblastoma are loss of heterozygosity (LOH) for the short arm of the chromosome 1 (1p), over expression of *N-myc* proto-oncogene, triploidy or tetraploidy, and defects in expression or function of nerve

growth factor receptor (NGFR). In our study, chromosomal analysis was carried out in the bone marrow sample of 4 years old boy following unstimulated cell culture technique. Conventional G-banding analysis of 40 cells revealed multiple complex chromosomal rearrangements, 48,XY,t(1;15)(p22;q24),add(6q25),add(7q32)x2,t(11p;?),t(11q23),add(12q24),t(19q13;?),del(20q13),+mar in 98% cells. In addition one tetraploid cell was observed. In this case 1(p22) was not deleted but translocated to chromosome 15(q24). Presence of t(11q23) suggests the loss of critical heterozygous region. The above abnormality suggests advanced stage of the disease and will experience poor prognosis. However, most of the participating chromosomes and their break points could not be identified due to limited resolution of the technique. Hence, it has been understood that cytogenetic study could form the primary step for understanding the disease and could further guide for employment of molecular techniques for analysis of amplification and/or deletion of other genes.

#### P02.171

#### Cytogenetic findings in one case of Nijmegen Syndrome developing Burkitt lymphoma

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Here we report an 8 years old girl who had post natal growth deficiency, microcephaly, facial dysmorphism, partial syndactyly of the second and third toes, susceptibility to infections, leucocytosis, immunodeficiency, adenopathy, but no sign of telangiectasia, ataxia and in evolution developed malignancy (Burkitt lymphoma). The first lymphocytes culture from this patient was unsuccessful, which is concordant with other reports from literature. It is known that is difficult to perform cytogenetic analysis due to the poor proliferation capacity of lymphocytes. From the first culture, 9 mitosis could be analysed, 6 metaphases had a normal karyotype while 3 were incomplete. The second culture revealed 16 mitoses, including four incomplete metaphases. Cells rearrangements of chromosomes were found in 4 metaphases. Chromosomes anomalies that we found are: isochromosome 11q, int del(7)(q21), acentric chromosomes and marker chromosome. The images of the metaphases were sent for reevaluation at Ulleval University Hospital Oslo, Department of Medical Genetics. By combining clinical manifestations and laboratory findings including cytogenetic findings and taking in account the evolution of the patient we sustain the diagnosis of Nijmegen Breakage Syndrome. At the moment of reporting this case, the diagnosis of Nijmegen breakage syndrome was confirmed by molecular analyses. Our patient had developed malignancy in a relative short period of time after she was first investigated and the prognosis is estimated to be poor. This is the first case of Nijmegen Breakage Syndrome reported in Romania.

#### P02.172

#### A child with proportional pre- and postnatal growth retardation, oculocutaneous albinism and normal intelligence with a 15(q26.2-qter) deletion

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We report on an 8 ½ years old girl with severe pre- and postnatal growth retardation, congenital heart malformation, facial asymmetry, oculocutaneous albinism, and subluxation of the radial heads. Postnatal growth was severely retarded and proportionate (height -4.5 SD, OFC -3SD at 8 years of age). Her intelligence was in the low normal range. Extensive endocrinological studies showed a normal growth hormone response to glucagon, without high growth hormone levels possibly indicating growth hormone resistance. At 7 years of age oculocutaneous albinism was diagnosed. The albinotic phenotype was unusual because, despite clear ocular and systemic features of albinism, the girl did not have foveal hypoplasia, misrouting, or nystagmus. By GTG-banding a deletion of band 15q26 was found. Array-CGH, using a 3783 BAC array, revealed a segmental monosomy of the 15(q26.2->qter) region, which was narrowed down to a 6.87 Mb deletion by us-

ing the Illumina Infinium 317 K SNP array system, and subsequently confirmed by fluorescence in situ hybridisation (FISH) analysis. The deletion appeared to have arisen de novo. The *IGF1R* (insulin-like growth factor 1 receptor) and the *NR2F2* genes were situated within, but the *OCA2* (oculocutaneous albinism II) gene (formerly called the P gene) was located outside the deleted region. Clinical findings in our patient were compared with previously reported cases carrying terminal deletions of 15q26.2. This allowed us to expand the clinical phenotype of terminal 15q26.2 deletions and to indicate candidate genes for several phenotypic features.

#### P02.173

#### Array CGH with genomic DNA extracted from peripheral blood detects overrepresentation of 12p material in a boy with Pallister-Killian syndrome features

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Pallister-Killian syndrome (PKS) is a rare disorder characterized by multiple congenital anomalies. The cytogenetic hallmark change in PKS is the presence of a supernumerary isochromosome 12p (i(12p)), which is usually limited to skin fibroblasts. Here we report on a 11-year-old boy with clinical features suggestive for PKS. Amniocentesis performed elsewhere did not show any abnormality. At the age of 9 years he developed seizures requiring anticonvulsant treatment. When we saw the boy at age 11, he had a coarse face with hypertelorism, a broad nasal bridge, a highly arched palate, long philtrum and prominent lower lip. On the right forehead and chest were streaks of hypo- and hyperpigmentation. G-banding analysis of peripheral lymphocytes revealed a mosaic karyotype with a small metacentric supernumerary marker chromosome (SMC) in about 30% of metaphases. Array CGH using genomic DNA extracted from peripheral blood identified overrepresentation of 12p, however, several 12p-clones showed a balanced ratio profile, which was not compatible with an isochromosome 12p. Therefore, we further characterized the marker by forward painting with subtelomeric BAC-clones for 12p (GS-496A11) and 12q (12q-21K18). While both clones showed regular signals on both chromosomes 12, the marker had a signal on only one end. Hybridising the centromere specific probe for chromosome 12 both normal chromosomes 12 and the marker showed unique hybridization signals. At present, we are using additional clones to characterize this der(12) further. Our results suggest that array CGH is suitable for the detection of low level mosaics and may thus complement conventional banding analyses.

#### P02.174

#### Molecular cytogenetic study of the partial monosomy 21q

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**INTRODUCTION:** Partial monosomy 21 is rare and only a few cases have been reported so far. Furthermore, the extent of the deletions has not been determined in the majority of these cases, which makes phenotype-genotype correlation difficult. In this study we present a 1 year old girl with severe neonatal hypotrophy, microcephaly, mental retardation, large low-set and deformed ears, wide forehead, strabismus, massive nose, long philtrum, wide mouth with thin lips, micrognathia, pectus excavatum, spinal curvature and clubfoot.

**METHODS:** The patient was analysed using conventional cytogenetics, array CGH and FISH. Array CGH was carried out using a sub-megabase whole genome tiling path BAC array and the results were verified by FISH analysis.

**RESULTS:** Cytogenetic analysis of the patient and her parents revealed a de novo deletion of chromosome 21q22.11-qter. The deletion was further characterized by array-CGH which justified the borders of the deletion to 21q22.12-qter (chr21:35,860,000-46,940,000, hg17). In addition we detected a 0,9 Mb duplication on chromosome 17q12. Array CGH was also performed on the cytogenetically normal parents and the results showed that the duplication was inherited from the phenotypically normal father.

**CONCLUSION:** In the present study we analysed a patient with a terminal deletion of 21q22. The clinical manifestations of our patient are mostly consistent with previously reported cases. However, since the extent of the majority of these deletions has not been determined, though phenotype-genotype correlation is difficult. We therefore recommend that patients with chromosomal abnormalities diagnosed by conventional karyotyping should be reinvestigated by array CGH.

#### P02.175

#### Partial trisomy of chromosome 10q due to the paternal balanced t(10;22)(q24;p11)

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The distal part of the long arm of chromosome 10 trisomy is a well defined, but rare syndrome. This chromosomal abnormality is almost the result of an balanced translocation from the parents. In the present report, we present a family having a girl with balanced translocation, a healthy girl and a malformed female infant with the pure partial trisomy of the long arm of chromosome 10q (q24 leads to qter), resulting from an unbalanced segregation of a paternal balanced translocation t(10;22)(q24;p11). In this patient, physical examination showed to have abnormal phenotyp such as microcephaly, small nose, depressed nasal bridge, blepharophimosis, micrognathia, hypotonicity and growth retardation. These clinical findins are compared with the other previously described cases with the trisomy 10q in the literature.

#### P02.176

#### 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.

**Keywords:** Partial trisomy of 1q42, trigonocephaly, microcephaly,

#### P02.177

#### X chromosome centromere instability: an epiphomenon of ageing or of Alzheimer disease?

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Premature centromere division (PCD) as a cause of improper chromosome separation is found in ageing cells, Alzheimer disease patients, various chromosome instability syndromes and cancers. Gender plays an important role in the pathogenesis of AD and can influence the risk of developing AD. Is PCD a citogenetic marker of this disease, or only epifomenon of ageing? PCD phenomenon of metaphase chromosome

from peripheral blood lymphocytes were evaluated in 25 sporadic AD subjects (14 females and 11 males) and 25 healthy elderly subjects (14 females and 11 males). Multifactorial analyses of variance was used to assess the frequency of PCD in our samples in correlation to age (up to 65, including 65 years vs. over 65 years) and illness (AD patients vs. elderly controls).

A statistically significant difference was found in the number of metaphase with PCD, X chromosome comparing AD female and female controls ( $P = 0.02$ ) and in the number of total PCD, X chromosome in female sample ( $P = 0.04$ ). Our results showed no positive correlation with age concerning the X chromosome. These results point to a fact that the X chromosome is preferentially affected in AD cases of women and that the amplification of instability of the X chromosome in women are not precisely connected to age.

#### P02.178

##### Congenital Lower Lid Entropion with pericentric inversion 9(p13-q12) and deletion in Chromosome 10(q23-qter)

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Entropion is an inversion of the eyelid toward the globe. The lower eyelid is more frequently affected and depending on the underlying disorder. Entropion may be either unilateral or bilateral. Congenital entropion is an extremely rare disorder, usually involves the lower eyelids. It is often familial and seen more frequently in Asians. The possible causes for this condition include structural tarsal plate defects (horizontal tarsal kink syndrome) and shortened posterior lamella (tarsus, conjunctiva, eyelid retractors). It has been reported that congenital entropion is a part of a syndrome involving multiple systemic anomalies. A case of primary congenital upper eyelid entropion with cardiovascular, musculoskeletal, and central nervous system abnormalities and another with congenital heart defect has been reported. But, to the best of our knowledge there is no report describing the genetic background of the disease. We report a patient of congenital lower lid entropion and corneal opacity who was referred to us for cytogenetic analysis. GTG-banding of 50 well spreaded metaphases revealed an interstitial deletion in chromosome 10 and pericentric inversion of chromosome 9. Chromosomal analysis showed 46, XX (57%)/46,XX,del(10q23-qter)(10%)/46,XX,inv(9p13-q12)(33%) karyotype. Most publications suggest that pericentric inversion of chromosome 9 is a polymorphic variation and its clinical significance is uncertain. Thus our finding raises the possibility that the congenital lower lid entropion locus may be located on chromosome 10. This represents a severe manifestation of the disease. Finally, a workup of this finding is suggested and more cases of congenital lower lid entropion needs to be screened using cytogenetics

#### P02.179

##### Chromosome segregation in blastomeres from translocation carriers.

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Balanced carriers of reciprocal and Robertsonian translocations suffer from reduced fertility and are at increased risk of recurrent spontaneous abortions or chromosomally unbalanced offspring. During meiosis I, segregation of the translocated chromosomes and their normal homologues produces a variety of unbalanced gametes. Preimplantation genetic diagnosis (PGD) offers to the translocation carriers an alternative to prenatal diagnosis and a possible pregnancy termination. PGD allows not only selection of normal embryos but also provides unique information about the chromosome segregation.

Interphase FISH combining subtelomeric and centromeric probes was used to examine the blastomeres of 126 embryos from 6 Robertsonian (2 female/4 male carriers) and 12 reciprocal translocation carriers (6 female/6 male carriers). In Robertsonian translocation carriers, 39.3% normal and 53.6% aneuploid (25% trisomic and 28.6% monosomic) embryos were found. Each reciprocal translocation had a different segregation mode, but at least one balanced blastomere was found in each translocation. The lengths of translocated segments varied from 7.8Mb to 140.4Mb (4% to 73% of the chromosome involved). Alternate

segregation (chromosome balanced) was found in 31% blastomeres. In unbalanced blastomeres, 39.4% resulted from adjacent segregation, 15.5% from segregation 3:1 and 14.1% of blastomeres were haploid, polyploid or chaotic. No statistical difference between male and female rates of chromosome abnormalities in both Robertsonian and reciprocal translocation carriers was observed. Comparison of the length of translocated chromosome segment and the type of segregation revealed a trend for higher proportion of normal blastomeres when shorter chromosome segments were involved.

#### P02.180

##### Array CGH analysis (two novel deletions) in pigment dispersion syndrome

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Pigment dispersion syndrome (PDS) is an ocular disorder in which melanin granules from the iris pigment epithelium are distributed throughout the anterior segment on various ocular structures. The prevalence of PDS is 2.5% in whites, and 1.6% in black population. In half of cases, PDS progresses to pigmentary glaucoma. The etiology of PDS is not known. Only one locus for PDS has been identified at 7q35-q36. We report the first, a 34-year old, male with PDS and a balanced 10;15 translocation, revealed using GTG banding. Molecular cytogenetic breakpoints were located using fluorescence in situ hybridisation (FISH) analysis at chromosome 10cen and on 15q between D15Z alphoid DNA (CP5033) and SNRPN (DNA probes from Abbott-Vysis and Oncor). The proband's karyotype was interpreted as 46,XY ,t(10;15)(p11.1;q11.1). Array CGH analysis using the DNA sample of the patient was genotyped in duplicate with the HumanHap300-Duo Beadchip (Illumina). Array CGH analysis did not show altered DNA sequences in the breakpoints of the translocation, but revealed two novel deletions in 2q22.1 and 18q22.1. These two CNV regions are not previously described in the Database of Genomic Variants. We suppose that the coexistence of t(10;15) and PDS in our patient is a coincidence. However, the deletion in 2q22.1, where the gene LRP1B has been located, may play a major role in the dysembryogenesis of the eye and cause the disorder. As array CGH shows a number of the chromosomal alterations, it is important to use this molecular karyotyping in diagnostic laboratories.

#### P02.181

##### Polymorphic variants and phenotype correlation. Findings in a Brazilian reference center

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**Objective:** To describe the frequency of polymorphic variants such as inv(9), 9qh+, 16qh+ and satellite increase and associated phenotype in a clinical cytogenetics core in a Women's University Hospital.

**Methods:** A retrospective review of the laboratory databases identified all cases of polymorphic variants from January 2003 to December 2007. Clinical records were also evaluated.

**Results:** The total number of cases analyzed in 5 years was 797. Cytogenetic evaluation of prenatal diagnosis cases with abnormal ultrasound findings were the most frequent (n=602[75,5%]) and showed rates of 73,5% (n=443) of normal karyotypes, 21,8% (n=131) of abnormal karyotypes and 4,7% (n=28) of polymorphic variants. Out of 162 karyotypes in women who were evaluated for sex-related disorders, 67,3% (n=109) presented a normal karyotype, 23,5% (n=38) presented an abnormal karyotype and 9,2% (n=15) presented polymorphic variants. Individuals who were investigated for recurrent abortions showed a 9,1% rate (n=3) of polymorphic variants among 72,7% (n=24) of patients with normal karyotype and 18,2% (n=6) with abnormal karyotypes. Abnormal karyotypes included both structural/numerical and autosomal/sexual abnormalities. Phenotypes associated with polymorphic variants at prenatal diagnosis were cardiac and facial

abnormalities followed by abdominal wall defects, central nervous system and diaphragmatic hernia. Abnormal amniotic fluid volume was present in 6 cases.

**Conclusion:** Although polymorphic variants are considered to be silent abnormalities within karyotype analysis, our study showed elevated rates of those variants in our reference center if compared to the general population which might indicate a strong correlation between the onset of an abnormal phenotype and karyotype polymorphic variants.

#### P02.182

##### Chromosome bands specially affected in fishermen who participated in the clean-up of the Prestige oil spill

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**Context** The oil tanker Prestige wrecked and produced contamination of the coast of Galicia (Spain), in 2002. A great number of people participated in the cleaning-up tasks.

**Objectives** To evaluate potential genotoxic effects in lymphocytes of the fishermen exposed, we analyzed the presence of chromosome alterations.

**Methodology** We analyzed 50 clean-up work and 46 non-exposed fishermen from 800 fishermen that were previously interviewed about the details of their cleaning-up tasks. 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. The collection of the samples was performed between July 2004 and February 2005. Peripheral lymphocytes were cultured. Breakpoints implicated in chromosomal abnormalities were identified by G-banding.

**Results** Comparison of cytogenetic data between the E and NE groups showed significant differences for: (i) the proportion of total chromosome lesions (E:67 lesions /4,521 metaphases; NE:35/4,859;  $P= 0.0079$ ), and (ii) the proportion of structural chromosome alterations (E:92 structural alterations/1,368 metaphases karyotyped; NE:32/1,285;  $P< 0.0001$ ). Statistical analysis of the 112 breakpoints implicated in these chromosomal abnormalities showed that seven bands were specially affected in the E group: 7p15, 7q33, 9q34, 13q32, 16p13.3, 18q23 and Xq21. In addition, in the NE group the bands most affected by the 60 breakpoints found were: 1q21, 11q23 and 14q23.

**Conclusion** Participation in clean-up tasks of oil spills may result in prolonged genotoxic effects lasting two years after exposure.

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#### P02.183

##### Primary ovarian failure associated with pericentric inversion of chromosome 3

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We report on a patient with primary ovarian failure and pericentric inversion of chromosome 3. Female was referred for genetic examination because of secondary amenorrhea. Menarche occurred at the age of 14 years; menses were irregular and stopped three years later. Patient had no additional clinical signs. At the age of 19 years she's height was 167 cm, weight 54 kg. Hormone tests revealed a high level of LH and FSH, with low level of estradiol (hypergonadotropic hypogonadism). Pelvic ultrasonography displayed the hypoplasia of uterus and ovaries; fallopian tubes were normal. Histopathology of gonads revealed dysgenetic ovarian tissue with single follicles. Her lymphocyte karyotype was 46,XX,inv(3)(p24.2;q21).

The patient's mother with similar inversion had irregular menstrual cycle. She was reported to have seven pregnancies, two of which have ended with births, and five pregnancies - medical abortions. The oldest sister of the patient was healthy and had regular menses. The karyotypes of she's eldest sister and father was normal.

A number of genes involved in female germ cells differentiation and ovarian cancer have been mapped in both short and long arms of the human chromosome 3. Probably, ovarian failure was resulted from this pericentric inversion.

#### P02.184

##### Prenatal diagnosis by QF-PCR: a case with partial trisomy of chromosome 13

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<sup>1</sup>Fondazione IRCCS, Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Milan, Italy, <sup>2</sup>Istituti Clinici di Perfezionamento, Ospedale Buzzi, Milan, Italy. QF-PCR is a well established method for the rapid prenatal diagnosis of the most common chromosomal aneuploidies (chromosomes 13, 18, 21 and sexual).

This technique provides fast results and it is coupled with the conventional cytogenetic analysis on samples of women with ultrasound abnormalities at different gestational weeks, failure of long term culture and in order to differentiate twins.

Here we report a case of pregnancy with ultrasound findings of multiple fetal malformations: QF-PCR performed on amniotic fluid didn't show numerical anomalies for chromosomes 18 and 21; it also identified the presence of two X chromosomes, associated to female sex.

About chromosome 13, the three specific chromosome polymorphic STR markers gave different results: D13S631 and D13S258 located in 13q31-32 and 13q21 respectively, suggested a possible trisomy, while the STR D13S742, located in 13q11-13q12.1, showed a normal chromosomal pattern.

The cytogenetic analysis confirmed the presence of an additional marker belonging to chromosome 13. Although we agree that QF-PCR cannot substitute the traditional cytogenetic analysis, this case brings out its ability to provide reliable and rapid results even in case of partial chromosomal aneuploidies of the examined chromosomes.

#### P02.185

##### Genomic instability in blood and fibroblasts submitted to controlled cell phone radiation levels.

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**OBJECTIVE** To evaluate genomic instability characterized by multiple targets of investigation both in conventional and molecular cytogenetics and apoptosis. The results comprise a 4-year joint project between a University and a Research Foundation.

**METHODS:** We have investigated the effects of AMPS, CDMA and GSM cell phone radiation in cultured blood samples and skin fibroblasts cell lines after radiation exposure in a specially designed exposition set up (TEM CELL) at SAR levels from 0,8 to 10W/kg. After the exposition, the cells were applied into different assays such as conventional cytogenetic analysis for structural and numerical abnormalities, micronuclei frequency, HER-2, C-MYC and TP53 gene status through FISH, CGH profile and apoptosis-related proteins.

**RESULTS:** Genomic changes such as chromosomal breaks, translocations, marker chromosomes and aneuploidy, when compared to the controls, were detected in SAR levels above 5W/kg as well as the increase of micronuclei frequency above 10W/kg. CGH profiles and FISH analysis did not show a significant difference between the controls and the exposed samples. It was observed a trend for the detection of different genomic instability processes above SAR limits of 5 and 10W/kg.

**CONCLUSION:** As genomic instability and chromosome damage are frequently related to tumorigenesis, our findings support the hypothesis of a dose-dependent positive effect of RF radiation on the genome of cells and also give further evidence to suggest continuous cytogenetic investigation of occupationally exposed individuals to SAR levels above the accepted international limits. Financial support: FUNTTEL/Brazil

#### P02.186

##### Radioinduced bystander effect revealed in vitro and in vivo in mixed human lymphocytes culture

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The new methodic approach to investigation of radioinduced bystander effect in somatic human cells with help of G-banding cytogenetics had been elaborated. The frequency of chromosome aberrations in proposed by us model system - "mixed human peripheral blood lymphocytes culture" consisted of cells differed on cytogenetic sex markers (XX, XY) and some morphological chromosome peculiarities had been studied. It had been shown that joint cultivation of intact lymphocytes

did not influence upon their background frequency of chromosome aberrations. Under joint incubation of X-irradiated *in vitro* (in doses 250 and 1000 mGy) and intact lymphocytes the dose-dependent interaction between targeted and untargeted cells had been discovered - cytogenetic effect in bystander cells (4.31 and 6.13 of aberrations per 100 metaphases, accordingly) cultivated with irradiated ones (6.99 and 21.19 of aberrations per 100 metaphases, accordingly) elevated their background level (2.27 aberrations per 100 metaphases). In mixed cultures established from unexposed donors and Chernobyl liquidators (with radiation doses in range 1010-2370 mGy) the mean-group frequencies of aberrations in irradiated cells were  $5.21 \pm 0.89$  per 100 metaphases, in bystander cells -  $4.05 \pm 0.64$  per 100 metaphases. The difference between spectrum of aberrations in exposed and intact cells had been revealed both *in vitro* and *in vivo* - in targeted cells specific cytogenetic markers of irradiation dominated (unstable and stable chromosome exchanges), as well as in "bystander" cells simple aberrations (chromatid breaks and terminal deletions that can be consider as the markers of chromosome instability) mainly induced. All cytogenetic effects had been characterized by essential interindividual fluctuations.

#### P02.187

##### **Can segregation analysis in spermatozoa of male carriers of a reciprocal translocation help in genetic counselling?**

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Balanced reciprocal translocations (Rcp), the most frequent chromosomal rearrangements in humans are mainly transmitted by one of the parents. We used the FISH technique to analyse the chromosomal content of spermatozoa from 20 Rcp carriers. A total of 15375 sperm nuclei were analysed. The mean frequency of normal/balanced and unbalanced gametes in the carriers was 47.4% (range 20.4-89.6%) and 52.6% respectively. In 11/20 carriers the frequency of normal/balanced and unbalanced sperm differed statistically ( $p<0.05$ ). In one carrier the frequency of balanced/normal sperm exceeded 75%. In literature, reviewed by Benet et al. (2005), 2/84 carriers showed also such a high %. In our series we observed 3/20 carriers who produced  $\geq 70\%$  of unbalanced sperm. On the other hand, in literature only one such carrier was found. Based on this information we can tell couples, with a male Rcp carrier and with normal fertility, that they have a chance of 50% at each conception of giving birth to a child with a balanced/normal chromosome complement. If however recurrent miscarriages keep occurring, sperm analysis may be offered in order to see if they belong to the carriers who produce  $\geq 70\%$  of unbalanced sperm. In this case PGD may improve their conception and delivery rate. In couples where the translocation carrier has fertility problems (e.g. OAT) PGD will probably increase their chance to become pregnant of a child with a normal/balanced karyotype. After repeated failures, however, sperm analysis may help these couples to decide to use rather donor sperm.

#### P02.188

##### **A balanced reciprocal translocation t(3;16)(q21;p13.3)**

##### **transmitted from mother to her offspring**

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We report a case with a maternal balanced translocation t(3;16)(q21;p13.3) detected by prenatal diagnosis at a male fetus. Prenatal karyotype from cultured amniocytes was performed due to increased risk for Down syndrome at serological test, without ultrasonographic anomalies.

For checking if the translocation in the child is de novo or inherited we performed chromosome analysis in the parents. Those are young and healthy, at the first pregnancy, achieved after a period of apparent sterility..

We have performed karyotypes of the parents and we found the same translocation in the mother. The breakpoints were established using GTG and QFQ staining methods. The karyotypes of the maternal par-

ents revealed a normal chromosomal constitution, suggesting a de novo rearrangement in the child's mother.

As there is obviously no genetic disease known in the child's mother, the translocation is apparently balanced and is high probability for the child not to suffer from a genetic disease caused by the translocation inherited from his mother. After genetic counseling the parents decided to continue pregnancy.

It is known that for the above translocation the total risk for an abnormal pregnancy is about 39% (33,33% abortion; 4,17% stillbirth or early death; 1,40% unbalanced offspring at birth) and the chance for an uneventful pregnancy and birth of a chromosomally normal or chromosomally balanced child about 61%. Due to the existing risk for birth of an unbalanced offspring we recommend an early chorionic villus sampling to perform chromosome analysis as soon as possible in a next pregnancy.

#### P02.189

##### **Case Report: Family reciprocal translocation (8;16)**

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Amniotic fluid sample from 32 year old pregnant woman was taken and send in our laboratory to perform prenatal cytogenetic diagnosis by standard techniques with GTG staining. Result was karyotype with reciprocal balanced translocation 46,XY,t(8;16)(p21.3;q22).

Parents were referred to our outpatient clinic for genetic counselling. They presented the following family history: two female children 8,5 and 6 years old with mental retardation, husband's brother and aunt are also mentally retarded. Blood samples were taken from both parents and chromosome analysis had been carried out on peripheral lymphocytes by standard techniques with GTG staining. Mother had normal female karyotype. Father had karyotype with the same balanced translocation as fetus. It was recommended to refer their two daughters and husband's brother for cytogenetic study.

Clinical findings of girls were: mental retardation and facial dysmorphism. Blood samples were taken from both girls for chromosome analysis by standard techniques with GTG staining and Fluorescence in situ hybridization. Karyotypes of both girls were pathologic with derivative chromosome 8, resulting from translocation (8;16) in father: 46,XX,der(8)t(8;16)(p21.3;q22)pat. It means that girls have partial trisomy of chromosome 16 and partial monosity of chromosome 8, which is the reason for their mental retardation.

#### P02.190

##### **Translocation (8;12)(p21;p11.2) found at Prenatal Diagnosis**

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The translocations are considered the most frequent chromosomal changes in the human species. Simple reciprocal translocations arises when a two-way exchange of material takes place between two non-homologous chromosomes. They can be "de novo" or familial, with a prevalence of 1:50000 and 1:500 births, respectively. The great majority of familial translocations between autosomes have no phenotypically effects.

We report a case of a 37-year-old pregnant woman with a normal obstetric history. Amniocentesis for cytogenetic evaluation was performed at 16 weeks of gestation.

Chromosome analysis of cultured amniocytes with GTL banding showed a apparently balance reciprocal translocation 46,XY,t(8;12)(p21;p11.2)mat - karyotype of parents were referred and demonstrated that the mother was carrier of the same translocation. FISH with painting probes confirmed the translocation.

Few chromosomal translocations that involve chromosome 8 and 12 were reported, but to our knowledge, all are related with other breakpoints. A bibliographic review is presented.

**P02.191****Reciprocal translocations 10;18 in a patient with miscarriages, menstrual and speech problems****A. Faraj Pour, C. Azimi;**

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Reciprocal translocations are usually an exchange of material between nonhomologous chromosomes. They are found in about 1 in 600 human newborns. Such translocations are usually harmless and may be found through prenatal diagnosis. However, carriers of balanced reciprocal translocations have increased risks of creating gametes with unbalanced chromosome translocations leading to miscarriages or children with abnormalities. Translocation between chromosomes 10 and 18 is very rare. A few reports have been published up to now including one in a patient with two miscarriages and another in a patient with juvenile neuronal ceroid-lipofuscinosis (Batten disease). Our case was a 24 year-old woman who was referred to our Department due to two miscarriages. She had also speech problems including stuttering and irregular menstrual periods. She has no problems in lips, teeth, jaw, nose, palate and hearing. Sonography of her uterus and ovaries was normal, and also her hormones were within normal range.

Family information revealed only stuttering in her uncle. Chromosomal analysis was made on her lymphocytes, using the standard banding techniques. Her karyotype was: 46, XX, t (10:18)(p11:p11).

**P02.192****Structural chromosome rearrangements in couples with recurrent spontaneous abortions****F. Farzanfar<sup>1</sup>, S. Morovvati<sup>2</sup>;**

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**Introduction:** Several studies have been done to determine the contribution of chromosome abnormalities in patient with recurrent spontaneous abortions. In this study we present a retrospective study of the cytogenetic data in 310 couples with recurrent spontaneous abortions registered in Imam Khomeini hospital in Tehran.

**Materials and Methods:** Giemsa-banding and Reverse-banding techniques according to standard procedures were routinely applied on peripheral lymphocytes in all patients.

**Results:** In our 310 couples with recurrent spontaneous abortions, the incidence of chromosomal rearrangements was 19 (6.1%). Of all chromosomal abnormalities detected, 42% (8/19) were balanced reciprocal translocations, 21% (4/19) were Robertsonian translocations, 16% (3/19) were para- or pericentric inversions, 10.5% (2/19) were X chromosome aneuploidies, and 10.5% (2/19) were marker chromosomes. Pericentric inv(9)(p11;q13) were excluded because they were considered as a normal population variant.

**Conclusion:** In this study we found chromosomal rearrangements in 6.1% of our patients with recurrent spontaneous abortions which is much higher than the incidence of chromosomal abnormalities in general population. Also in current study the prevalence of autosomal rearrangements in females was slightly more than males.

**P02.193****Clinical features of a case with ring chromosome 18****M. Zamanian<sup>1</sup>, F. Mahjoubi<sup>2</sup>;**

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Chromosome 18 Ring is a rare disorder in which there is loss (deletion) of genetic material from one or both ends of the 18th chromosome and joining of the chromosomal ends to form a ring. Associated symptoms and findings may vary greatly in range and severity from case to case, depending upon the amount and location of lost genetic material and other factors. A ring may also be formed without the loss of any genetic material.

Here we report an additional case of a 14 months girl with r (18). The girl was born at term after an uncomplicated pregnancy and delivery. Birth weight was about 1.5 kg, length 48cm, and head circumference

36cm. The girls presented hypertelorism, hypotonia, epicanthal folds, abnormal fingers, low set ears, and abnormally growth teeth. Echoardiography indicated dilation of the aorta.

Karyotyping after lymphocyte culture at the age of 14 months revealed 46,XX,r(18)(q21.2pter). The parent had normal karyotype.

The clinical feature of our case is mostly compatible with the other reported cases of r(18) except the presence of abnormal teeth and heart problem. This report further contribute to the clinical of the r(18).

**P02.194****A girl with ring chromosome 5****E. Dagyté<sup>1,2</sup>, L. Cimbalistienė<sup>1,2</sup>, V. Kučinskas<sup>1,2</sup>;**

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We report a girl presenting ring chromosome 5. The girl was born of the second pregnancy of healthy, 25 year old mother and 28 year old father. The pedigree is otherwise unremarkable with respect to stillbirths, malformations and mental handicap. The first pregnancy resulted in spontaneous abortion.

The girl was born at 37 weeks of gestation. Birth weight was 1895 g, length was 43 cm, head circumference was 29 cm and chest circumference was 26 cm. Dysmorphic features included microcephaly, upslanting palpebral fissures, hypotelorism, microstomia, high palate, micrognathia, short neck, hirsutism. Abdominal ultrasonography revealed no abnormalities.

Clinical examination at 5 years of age showed weight 11,6 kg (<3 %), length 90 cm (<3 %), head circumference 43 cm (<3 %). Dysmorphic features included microcephaly, upslanting palpebral fissures, microstomia, mild micrognathia, long smooth philtrum, narrow vermillion, neck webbing, low posterior hairline, short toes, broad thumb, mild hirsutism. Mental development was normal. Cytogenetic analysis of peripheral blood lymphocytes revealed 46,XX,r(5)(pterq35) karyotype. Both parents had a normal karyotype. Cytogenetic analysis was performed from GTG banded metaphases.

**P02.195****Observation of satellite association in couples with history of habitual abortion****F. Manoochehry<sup>1</sup>, F. Mahjoubi<sup>1,2</sup>, S. Abdollahy<sup>1</sup>;**

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Chromosomal aberrations account for approximately 50% of fetal losses prior to 15 weeks. Human acrocentric chromosomes are frequently found in association. This satellite association has been reported to be random by some researchers. However, there are some new reports of possible association between this phenomenon and idiopathic recurrent abortions. In our institute in order to identify the role of chromosomal rearrangements in aetiology of habitual abortions, we analyzed chromosomes of some couples with the history of miscarriages. Chromosomal karyotyping analysis was performed on cultures of peripheral blood lymphocytes by using the GTG-band method. Sixteen metaphase cells were analyzed for their chromosome constitution in each sample. We found that in 4 patients with idiopathic recurrent abortion the incidence of satellite association is quite high in each cells and in overall. Thus the observation in the present study shows that there might be possible association between satellite associations and recurrent miscarriages at least in subpopulations of the patients.

**P02.196****Molecular Cytogenetic Characterization and Genetic Counseling in Some Rare Cases of Sex Chromosomes Abnormalities****A. M. Mohamed<sup>1</sup>, A. K. Kamel<sup>1</sup>, I. Mazen<sup>1</sup>, N. Dessouki<sup>2</sup>, H. A. Hussein<sup>1</sup>, M. O. El -Rouby<sup>1</sup>;**

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We reported on six cases with rare sex chromosomal abnormalities. Our aim was to use molecular cytogenetics to correlate karyotype findings to phenotype for proper genetic counseling. Two cases pre-

sented with ambiguous genitalia. The 1<sup>st</sup> case was reared as male and had right palpable gonad, uterus and left streak gonad. Its karyotype was 45,X/45,der(X)/46,X,der(X).ish der(X)t(X;Y)(p22.3;p11.3)(DXZ1+,DYZ3+,SRY+,KAL+,telXp-/Yp-). The second case reared as a female and had right testis, a uterus and left ovary with some follicles. The karyotype was 45,X/46,X,+mar.ish idic(Y)(qter→p11.3::p11.3→qter)(wcpY+,DYZ3++,SRY+,telYp-). The 1<sup>st</sup> case diagnosed as mixed gonadal dysgenesis and the second as true hermaphroditism. To our knowledge this is the 1<sup>st</sup> case of true hermaphroditism with this distal break points. The other 4 cases presented with primary amenorrhea and short stature. Karyotype of 3<sup>rd</sup> case was 45,X/46,X,+mar. ish idic(Y)(pter→q11.1::q11.2→pter)(DYZ3++,SRY++). The 4<sup>th</sup> case had 46,X,der(X) ish. Der(X)(pter→q21.1::p22.1→pter)(DXZ1+,XIST+,telXp+,STS++), the 5<sup>th</sup> case had severe short stature and her karyotype was 46,X,der(X). ish der(X)(qter→p11.2::p11.2→qter)(wcp X+, DXZ1++). The 6<sup>th</sup> case had 46,X,der(X).ish idic(X)(pter→q25::q25→pter)(wcpX+DXZ1++,XIST++,telXp++).

A team of specialists collaborated together to give the proper genetic counseling and follow-up specially for cases with Y chromosome who had the risk of gonadoblastoma. We recommend further investigations using Array CGH for identification of duplication / deletion in different regions of sex chromosomes.

#### P02.197

#### Molecular cytogenetic characterization of a small supernumerary marker chromosome derived from chromosome 11 in a mother and a child with distinct phenotypes

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Small supernumerary marker chromosomes (sSMC) are defined as structurally abnormal chromosomes that cannot be identified or characterized unambiguously by conventional banding cytogenetics alone, and are generally equal in size or smaller than a chromosome 20 of the same metaphase spread. sSMC derived from chromosome 11 are rare and, so far, it is not yet clear which regions of chromosome 11 are critical and have clinical consequences.

We report a mother and a child with a karyotype 47,XX,+mar[21]/46,XX[9] and 47,XY,+mar[25]/46,XY[5], respectively. After centromeric FISH the origin of the sSMC was ascertained as derived from chromosome 11. Subcentromeric FISH revealed the presence of two different shapes of the sSMC in the mother, with apparently the following breakpoints - ish r(11)(::p11.12~11.2->q12::)[2]/min(11)(p:11.12~11.2->q12::)[7]; and three different shapes of the sSMC in the child - ish r(11)(::p11.12~11.2->q12::)[6]/r(11;11)(::p11.12~11.2->q12::p11.12~11.2->q12::)[3]/min(11)(p:11.12~11.2->q12::)[4]. The sSMC was microdissected and amplified by DOP-PCR. The amplified DNA was analysed by array painting, using fullfilling array specific for chromosome 11 (with 100-150 kB resolution). An unexpected result revealed an unusual sSMC. A maternal uniparental disomy of chromosome 11 was excluded. The child presents a more severe phenotype, with facial dysmorphisms, strabismus, ptosis, mental retardation and developmental delay. The mother has a congenital cardiopathy and no apparent mental retardation.

Molecular cytogenetics techniques are a valuable tool for the accurate identification of the genetic origin and content of sSMC, contributing to a better genotype/phenotype correlation.

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#### P02.198

#### Three de novo deletions, one transposition, and one inversion of chromosome 6 in a patient with complete absence of expressive speech and reduced pain perception

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Using the Infinium HumanHap300 Genotyping BeadChip SNP array and BAC-based FISH we found three adjacent, but non-contiguous

de novo deletions, one transposition and one pericentric inversion of a chromosome 6 in a patient whose karyotype was previously described by banding analysis as 46,XX,del(6)(q13q15),inv(6)(p11.2q15). In addition to the deletion of band 6q14 detected previously by classic cytogenetics, we found two additional microdeletions. The most distal one is a 360 kb deletion in band 6p12.3, containing the genes *RHAG*, *CRISP1*, 2, and 3, and *PGK2*. The second deletion in 6p12.2-p12.1, containing the genes *PKHD1*, *IL17*, *MCM3*, *EFHC1*, and *TRAM2* genes is 1.15 Mb in size. The deletion in region 6q14.3-q16.1, reported previously, was mapped more precisely and determined to be 11.9 Mb in size. It contains 27 genes, some of which are involved in pain sensation, growth regulation, and tissue modeling during development. The refined karyotype is 46,XX,der(6)(pter<sup>0</sup>p12.3::p12.1<sup>0</sup>p12.1::q14.3<sup>0</sup>p12.1::p12.3<sup>0</sup>p12.2::q16.1<sup>0</sup>qter) or (pter<sup>0</sup>p12.3::p12.1<sup>0</sup>p12.1::q14.3<sup>0</sup>p12.1::p12.2<sup>0</sup>p12.3::q16.1<sup>0</sup>qter). The main clinical features of this 31-year-old women are dysmorphic facial features consisting of a broad face, prominent glabella, broad nose, and hypertelorism, non-progressive deficit of motor control, in particular a broad-based slow-motion-like gait, absence of speech development, inability to acquire and to comprehend theoretical knowledge, and reduced sensitivity to pain. The rearrangement of chromosome 6 presumably originated in a paternal meiosis. By combining the SNP array and FISH data we were able to completely map and to reconstruct this highly complex rearrangement in a single chromosome.

#### P02.199

#### Characterization of a small supernumerary marker chromosomes using cytogenetic and molecular cytogenetic methods (two case reports)

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Small supernumerary marker chromosomes(sSMC),defined as additional abnormal chromosomes, too small for characterization by conventional banding cytogenetics alone,are present in 0.043% of newborns and 0.077% of prenatal cases.About 1/3 of the sSMC are correlated with a specific clinical picture,while most of the remaining sSMC have not yet been associated with clinical syndromes.

We present two cases with sSMC in karyotype which were initially studied by GTG banding technique.To define origin of sSMC,additional molecular cytogenetic methods (FISH) were performed using probe sets:cenM-FISH and subcenM-FISH Mix for the corresponding chromosomes.

Patient 1 had a diagnosis of total anomalous pulmonary venous return and facial dysmorphism.Here a mosaic karyotype 47,XX,+mar[22]/46,XX[11] was detected.The sSMC was characterized as an invdup(22)(q11.21).Similar cases were previously reported and the patient could now clinically be diagnosed as having a Cat-Eye syndrome.

Patient 2 was a prenatal diagnosis of 42-years old woman with fetal karyotype 47,XY,+mar(de novo).In this case sSMC was characterized as an invdup(15)(q11.1).According to the literature similar cases didn't show any clinical abnormalities.This type of sSMC is the most common (appears with 30% frequency).

It is difficult to predict precisely the phenotypic risk that could be associated with the presence of sSMC.The origin of sSMC is important factor in determining its possible phenotypic effect.Conventional cytogenetic techniques are limited.Molecular cytogenetic methods are necessary in terms of providing the information about marker origin and structure.It's only way to establish phenotype-karyotype correlation and discuss the results in genetic counseling.

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#### P02.200

#### The Effect of Chromosomal Rearrangements on Gene Expression

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The t(11;22)(q23;q11) is the only recurrent non-Robertsonian translocation in humans. Carriers are phenotypically normal, but at risk of having progeny with Emanuel syndrome. It is conceivable that this large chromatin rearrangement influences the transcription levels of genes mapping both near, and distant, to chromosome breakpoints. To test this, we compared gene expression profiles of lymphoblastoid cell lines from 13 cytogenetically normal individuals, to those of 9 balanced translocation carriers and 4 unbalanced Emanuel syndrome patients. Comparison of unbalanced individuals with controls revealed twice as many gene expression changes than the balanced/control comparison. This was anticipated as individuals are partially aneuploid for both HSA11 and 22 and are phenotypically affected. Consistently, a statistically significant fraction of the differentially expressed genes mapped to these two chromosomes.

Permutation tests showed that, despite being lower, the number of differentially expressed genes between the two groups with complete genome complements is significantly higher than that observed when one compares control samples alone. This suggests that balanced rearrangements have a greater effect on gene expression than normal variation even though individuals are phenotypically normal.

Interestingly, in balanced individuals, expression of HSA11 genes is affected to a much higher degree than those on HSA22. We hypothesize that the position of the derivative chromosomes is altered within the nucleus, with derivatives chromosomes being moved to a position comparable to the normal HSA22. This would result in HSA11 genes being placed into an altered chromatin environment, thus altering their expression. We are currently investigating this possibility.

#### P02.201

#### New report of an unbalanced Xp/Yq translocation detected in a mentally retarded patient with short stature and ichthyosis

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Deletion of Xp22.3 causes different X-linked disorders in males; in females, depending on the X-inactivation patterns or gene disruptions, the phenotype may be variable (Schinzel, 2001). Patients with deletions in this region have been previously reported in a restrict number of cases, and presented with distinct combinations of usually non-related clinical manifestations such as short stature, mental retardation, ichthyosis, chondrodysplasia punctata, attention deficit hyperactivity disorder, ocular albinism and epilepsy (Lonardo et al., 2007). The phenotype is correlated with the deletion breakpoints on the X chromosome and the respectively affected genes. As a result of gene sequence similarities between Xp22 and Yq11, homologous recombination between these two regions has been suggested as the cause of X/Y translocations.

The authors report on a male patient aged 17 presenting with mild mental retardation, short stature, X-linked ichthyosis and with steroid sulphatase deficiency. High resolution GTG, QFQ and CBG banding revealed the following karyotype: 46,Y,der(X)t(X;Y)(p22.3;q11.2)mat. The study of the parents proved that the translocation was of maternal origin. PCR-based techniques positioned the centromeric breakpoint between genes VCX2 (Xp22.31) and AMELX (Xp22.2). The analysis of the DYS385 marker, located in Yq11.21, suggested that this region is duplicated in the patient.

A review of cases described in the literature with similar cytogenetic findings will be equally addressed and compared with the present case. The authors highlight the importance of careful evaluation of Xp22.3 deletions in terms of clinical prognosis, follow-up and genetic counselling.

#### P02.202

#### Telomere length and Random Aneuploidy in Hepatitis C patients

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Hepatitis C virus (HCV) is one of the major causes of hepatocellular carcinoma and has recently been recognized to play a role in the pathogenesis of B-cell lymphoma. Random aneuploidy is one of the genetic instability parameters observed in cancer. Telomeres are the end structures of each chromosome. These structures protect from shortening at each replication cycle. Telomerase is the enzyme which

is responsible for the elongation of the telomeres, and exists only in cancer, stem and progenitor cells. A reduction in telomerase levels was observed in HCV patients lymphocytes compared to healthy individuals.

The aim of this study was: 1) Evaluating random aneuploidy rate in lymphocytes of chronically infected HCV patients compared to individuals who eradicated the virus (remission group) and to healthy controls. 2) Developing new method for measuring relative telomere length.

For random aneuploidy, we applied the FISH technique with probes from chromosome 9 and 18. For the telomere detection we used the PNA telomere kit.

A significantly higher random aneuploidy rate was found in the HCV group as compared to the control group, while the remission group had intermediate rates between the other tow groups.

We observed significantly shorter telomere length in HCV patients compared to the controls and to the group in remission. We found a reverse correlation between shorter telomeres and the rate of aneuploidy.

Random aneuploidy and short telomeres are two co-existing biological phenomena found in HCV patients; it may represent pre malignant changes which could play a role in the cascade leading to neoplasia.

#### P02.203

#### A new candidate gene for mental retardation and severe central hypotonia

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We report a female patient affected by mental retardation and severe central hypotonia. At birth, the patient had normal parameters. The first clinical signs were noticed during the first months of life when she was unable to hold her head.

At first examination, CT scan, muscle enzymes, metabolic parameters (including amino acids and organic acids chromatography), mucopolysaccharides and thyroid were normal. At one year of age, she showed major axial and peripheral hypotonia and she was not able to hold her head. She was not able to sit and has no tendon reflexes. She displayed abnormal and permanent choreic movements. She does not suffer from epilepsy. Nerve conduction velocity and electromyogram are normal. She is not deaf and her EEG and MRI are normal. At age 3, her OFC is normal, she shows major hypotonia and she is still unable to hold her head or to roll-over and suffers from oculomotor dyspraxia. She has no motor autonomy. She can speak 3-4 words and has a very good eye contact.

Routine cytogenetic analysis revealed an apparently balanced de novo t(10;13)(p14;q13) translocation. The molecular characterization of the chromosomal rearrangement shows that a gene is interrupted on chromosome 13. This gene encodes a protein of unknown function. Preliminary analysis reveals that it is only expressed in brain and testis, making it a good candidate for the neurological phenotype of our patient. However, more patients need to be screened to confirm its implication in this new phenotype.

#### P02.204

#### De novo t(1;2)(q25;q21) case with various dysmorphic features and mental retardation

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Translocation is the most clinically significant abnormality of all the structural chromosome rearrangements. Balanced de novo chromosome rearrangements may be found in patients with mental retardation; however their role in the developmental phenotype of the patient is usually unclear. Loss of genetic material within the breakpoint regions may be responsible for the phenotype.

We describe a 9-year-old boy with various facial dysmorphic features such as telecanthus, epicanthic folds, upslanting palpebral fissures, long eyelashes, hypoplastic alae nasi, thin upper lip and high palate. In addition, mild mental retardation, developmental delay, bilateral fifth finger clinodactyly, valgus deformity in the second toe, skin syndactyly between the second and third toes and pes planus were also present. Because of the diagnosis of mental retardation and the physical ab-

normalities, cytogenetic study was performed. Balanced translocation t(1;2)(q25;q21) was detected. His parent's karyotypes were found to be normal. In the literature no similar breakpoint region has been reported. Here we point out the importance of this rare translocation that may provide a valuable clue to the phenotypic findings and identification of target loci.

#### P02.205

##### Possible post-meiotic origin of the constitutional t(11;22)

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The constitutional t(11;22) is the only known recurrent non-Robertsonian translocation in humans. The translocation breakpoints occur within palindromic AT-rich repeats on chromosomes 11q23 and 22q11. In our previous studies, we established translocation-specific PCR by using the sequence of the translocation junction fragments from both derivative translocation chromosomes. Using this method, we successfully detected de novo t(11;22)s in sperm samples from normal healthy males, but not in lymphoblasts or fibroblasts. To understand how this translocation occurs during spermatogenesis, we divided sperm samples into small aliquots prior to DNA extraction and directly performed translocation-specific PCR. Multiplex PCR allowed us to detect der(11) and der(22)-specific PCR products of de novo origin, which were amplified concomitantly from the same aliquots. This result suggests that the de novo t(11;22) occurs as a reciprocal translocation. Further, we changed the combinations of primer pairs, which allowed us to identify dicentric and acentric translocation derivative chromosomes. Interestingly, these two unusual derivative chromosomes also appear concomitantly in the same aliquots. Based on the fact that almost no unbalanced translocation products were identified, we speculate that de novo t(11;22) translocations are likely to arise at post-meiotic stages of spermatogenesis.

#### P02.206

##### A rare translocation (15;16)(p10;p10) in a normal man

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Whole-arm translocations result from centric fusion of two chromosomes (usually nonhomologous), followed by a reciprocal exchange with fusion of entire arms in two derivatives, each with a hybrid centromere. Nevertheless, there are at least two alternatives: that both points of interchange will be juxtapacentromeric and then each derivative will conserve its own whole centromere, or that one point will be centromeric and the other juxtapacentromeric, so that one derivative will have a hybrid centromere and the other will conserve its original but reduced centromere. Although the underlying mechanisms are still unknown, detailed molecular analyses have provided evidence that centromere duplication may predispose to constitutional centric fusion and consequently favour the occurrence of whole-arm translocations. Constitutional whole-arm translocations are rather rare and can be identified in either a balanced or unbalanced state. Most individuals with balanced forms have a normal phenotype, but may be subfertile. In contrast, patients with unbalanced whole-arm translocations have abnormal phenotypes. Here, we describe a 34-year-old healthy married man who was referred because of consanguinity. His wife and first child were healthy and they wished to have a second child. Chromosomal studies were carried on lymphocyte cultures using G-banding. All mitoses showed a reciprocal whole-arm translocation (15;16). The karyotypes of his wife and his child were normal. Other family members were not available for study. Literature review confirmed that t(15;16) is very rare.

#### P02.207

##### A rare de novo translocation (18;21)(q23;q11.2) in a patient with Down syndrome

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We report a 8-year-old boy with clinical features of Down syndrome: small stature, brachycephaly, flat occiput, small nose, low nasal bridge, upslanting palpebral fissures, inner epicanthal folds, Brushfield's spots, anomalous auricles, dental hypoplasia, short broad hands, Simian crease, hypoplasia of midphalanx and clinodactyly of fifth fingers, wide gap between first and second toes, and mental deficiency. Karyotyping of this patient showed: t (18;21)(q23;q11.2). Cytogenetic studies on his parents and his only sister revealed normal karyotypes. A few reports have been published concerning familial subtelomeric translocation of chromosomes 18 and 21, but we could not find any de novo translocation of 18/21 similar to our case in the literature.

#### P02.208

##### A comparison of maternal age among fetuses with trisomy 13 and trisomy 18

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Objective: To evaluate the difference of maternal age among fetuses with trisomy 13 and trisomy 18.

Methods: A retrospective review of the cytogenetic laboratory databases identified all cases of prenatally diagnosed trisomy 13 and trisomy 18 from January 1997 to December 2007, and maternal age was recorded. Ages were compared by unpaired t test, with a power of 44.6% because the two groups had no normal distribution. A "p" value < 0.05 was considered statistically significant.

Results: 61 affected fetuses were included: 38 cases of trisomy 18 and 23 cases of trisomy 13. The mean maternal age in cases of trisomy 13 was 30.3 years (16-44 years, sd = 7.8 years), and in cases of trisomy 18 was 29.9 years (16-46 years, sd = 9 years). The difference in mean maternal age between these two groups shown a "p" value = 0.82. The frequency of patients younger than 35 years of age for trisomy 18 was 65.7% (n=25) and for trisomy 13 was 65.2% (n=15).

Conclusion: Our study showed that the difference in mean maternal age among fetuses affected by trisomy 13 and trisomy 18 was not significant. It was shown a trend of more cases diagnosed in patients younger than 35 years old. However, more data are needed to establish whether this is a true phenomenon or a bias of the population studied.

#### P02.209

##### A case of de novo trisomy 12p

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Research Centre for Medical Genetics of RAMS, Moscow, Russian Federation. We are reporting the clinical and cytogenetic studies on a 6 months-old boy who was referred for karyotyping because of distinct craniofacial dysmorphic features. Phenotype of proband included: acrocephaly, flat occiput, mid-face hypoplasia, upslanting palpebral fissures, hypoplastic supra-orbital ridges and high narrow palate, dysplastic ears, brachydactyly, broad thorax and pectus excavatum. Psychomotor and mental retardation was marked. Conventional cytogenetic analysis of patient using GTG-banding revealed 47,XY,+mar karyotype. Supernumerary marker chromosome (SMC) resembled 21q or 12p and had AgNOR on short arm. The karyotypes of parents were normal. FISH analysis with LSI 21(21q22.13-q22.2) and WCP 12 DNA-probes (Vysis, Abbott) and DAPI counterstaining was performed. SMC was LSI 21-, WCP12+. FISH analysis showed that SMC was composed of chromosome 12p and short arm of one of acrocentric chromosomes. Inverted DAPI-staining revealed that short arm of the SMC contained only heterochromatic DNA and NOR of acrocentric chromosome. Therefore we proposed that this part of SMC has not a pathological phenotype effect. An adverse effect on the phenotype is caused of a de novo trisomy 12p. Probably this chromosome is a result of parental meiotic mutation. Use of FISH with high-specific DNA-probes increases the quality of cytogenetic diagnosis and allows one to base further recommendation for genetic counseling

**P02.210****Clinical features of a case with trisomy 10q and monosomy 3p resulting from a maternal balance translocation: A case report and review of clinical features**

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Here we describe clinical and cytogenetic data on 2 year female child with partial trisomy for the distal part of the long arm of chromosome 10 (10q22->qter) and a concomitant monosomy 3(p25->pter) as a result of a maternal balanced reciprocal translocation. Her karyotype was ascertained as 46,XX,der(3)t(3;10)(p25;q22). The father had normal karyotype. The mother had an apparently balanced translocation involving chromosome 3 and 10 [46,XX,t(3;10)(p25;q22)]. To our best knowledge, this is the second reported case of partial trisomy 10q and partial trisomy 3p and first reported case from Iran. Clinical features of this case and a few published cases will be reviewed briefly.

**P02.211****Partial duplication of chromosome 16p: narrowing of the critical region responsible for the common phenotype**

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Clinical descriptions of almost 50 patients with trisomy 16p, encompassing the band 16p13.3, have been reported to date in literature. Phenotypical features commonly associated to this chromosomal alteration are: severe developmental delay/psychomotor retardation, growth retardation, seizures, microcephaly, cleft palate, mild flexion contractures, clubbed feet, genitourinary abnormalities, congenital heart defects, a specific facial appearance and, usually, a poor prognosis.

We report the case of a female patient (aged 16 years) with moderate to severe mental retardation in which we disclosed, by the means of array-CGH (BAC-array at a resolution of 1 Mb) a cryptic tandem duplication of chromosomal region 16p13.3, sized about 2 Mb.

The main clinical features were: psychomotor retardation, very limited speech, relative microcephaly, narrow forehead, deep set eyes, narrow palpebral fissures, wide nasal bridge, long philtrum, rounded nasal tip, thin upper lip, midfacial hypoplasia, a protruding mandible, dysmorphic and low-set ears, tapering fingers, clubbed feet. The patient never had seizures.

Our patient's duplication is the smallest one reported to date with clinical features that resemble very closely the characteristics of larger 16p trisomy cases. We highlight, however, the relative mildness of our patient's phenotype.

We suggest that genes included in this 2 Mb region on 16p13.3 should be responsible of the main pathological findings of "trisomy 16p syndrome", such as mental retardation, typical facial appearance, microcephaly, hands and feet abnormalities.

Remarks about the possible causative role of different gene mapped in the region and comparisons with other microduplication phenotypes mapped on other regions on chromosome 16p are made.

**P02.212****Prenatal findings: a foetus with trisomy of 22 chromosome**

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Trisomy of chromosome 22 is very rare chromosomal pathology, particularly in the second or third trimester of pregnancy. We present a 33-year old para I, gravida IV woman. She was consulted at 12 weeks of gestation due to aggravated obstetric history. Her previous pregnancy was terminated at 19 weeks of gestation due to fetal meningocele. After three years patient delivered a normal boy. The recurrent risk for meningocele was about 2%. For other congenital malformations risk was the same as population risk. Fetal NT and maternal serum biochemistry (PAPP-A and β-hCG) was assessed at 12 weeks of gesta-

tion. NT thickness was 2,92 mm (1,95 MoM), PAPP-A was 0,31 MoM and β-hCG was 0,6 MoM. The combined risk for trisomy 21 was >1:50 and for trisomy 18 1:61. The ultrasound scan at 16 weeks showed asymmetrical fetal growth restriction and congenital heart disease - tricuspid valve atresia, pulmonary atresia, hypoplastic right ventricle. The parents opted for termination of pregnancy at 20 weeks of gestation.

The amniocentesis was performed for quantitative fluorescent polymerase chain reaction and karyotype analysis. QF-PCR tests results were negative for chromosomes 13, 18 and 21 trisomies. Chromosome analysis of lymphocytes revealed 47,XY,+22 karyotype. Fluorescent in situ hybridization (FISH) was performed using microdeletion probe localized to 22q11.2. FISH analysis confirmed the trisomy of chromosome 22 in the proposita. In view of these results parental chromosome analysis was not requested.

**P02.213****Complete trisomy 5p due to paternal transmission of chromosome 5 centric fission products**

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In humans stable centric fission of chromosomes are rare event, and only few families are reported with this chromosomal aberration. To our opinion this is the first report of centric fission of chromosome 5 in several generations. Complete trisomy 5p also is very rare chromosomal abnormality. Clinical findings of trisomy 5p are macrocephaly, facial dysmorphism, hypotonia, tracheobronchial abnormalities and heart defects. We describe a newborn boy was born in 40 weeks of gestation from the third pregnancy as second child in the family. The first child was healthy girl, and the second pregnancy ended with spontaneous abortion during 10 weeks of gestation. Birth weight was 3700 g, height 54 cm, HC - 40 cm. After delivery hypotonia, some respiratory difficulties, cephalohematoma in parietooccipital region were observed. All sutures of head were widely opened. Phenotypically the patient had low situated ears, short palpebral fissures, a single crease. The patient died from respiratory infection at 5 month.

Routine chromosome analyses (GTG-banding) were performed on peripheral blood lymphocytes of proband, his parents and grandparents. The karyotype of proband showed complete telocentric trisomy 5p: 47,XY,+der(5)(pter→p10:). The cytogenetic analyses of father and his mother (proband's grandmother) revealed centric fission of chromosome 5, thus the karyotypes was 47,XY,-5,+fis(5)(p10),+fis(5)(q10) and 47,XX,-5,+fis(5)(p10),+fis(5)(q10), respectively.

**P02.214****Atypical Turner syndrome due to structural chromosomal rearrangements - clinical and cytogenetic study of 9 patients**

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Turner's syndrome is the most common chromosomal abnormality in females, affecting 1:2,500 live female births. It is due to the total or partial absence of an X chromosome in a female. The most consistent clinical features are short stature and ovarian failure. A variety of chromosomal abnormalities are associated with Turner syndrome.

We present the cases diagnosed in Iasi Medical Genetics Center between 2000-2008 with atypical Turner syndrome due to structural chromosomal rearrangements, in order to discuss cytogenetic diversity of Turner syndrome. Cytogenetic study was performed using peripheral lymphocytes with G banding.

Between 2000-2008, our Cytogenetics lab has performed 1380 karyotypes, 27 being recorded with the diagnosis of Turner syndrome. Out of these, 9(33.3%) were due to structural chromosomal rearrangements. Abnormalities identified were: iXq ( 7cases) and r(X) (2cases).

Clinical and cytogenetic data are compared with literature data for each case. Typical and particular features are discussed.

In conclusion, we present 9 cases diagnosed with atypical Turner syndrome due to structural X chromosome abnormalities, in order to illustrate some rare variants and to discuss particularities and cytogenetic diversity of Turner syndrome.

**P02.215****Cytogenetic analysis of Turner syndrome**

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Turner syndrome is caused by the absence of all or part of the second sex chromosome. In our study on 100 female patients clinically diagnosed with Turner syndrome:

46 patients were 45,X

10 patients were mos 45,X/46, XX

2 patients were mos 45,X/47, XXX/46,XX

8 patients were mos 45,X/47, XXX

31 patients had a structurally abnormal X chromosome (mainly iso-chromosome, deletion p or q arm, ring X chromosome) and 2 patients had a structurally changed Y chromosome

The phenotype is variable and includes short stature and gonadal dysgenesis. Mental retardation is not a feature of Turner syndrome.

Conventional cytogenetical G-banding method and Fluorescence in situ hybridization technique were considered in cultured peripheral blood.

Postnatal recognition of the syndrome requires genetic counselling of parents and supportive multidisciplinary treatment.

**P02.216****The importance of women karyotyping in assisted reproduction**

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**INTRODUCTION:** Recent researches show that the infertility is related to both male and female genetics. Patients, who applied to our center, evaluated according to their indication and karyotypes.

Karyotype analysis was applied for the patients with the indication such as; recurrent implantation failure (RIF), recurrent pregnancy lost (RPL), male factor and abnormal fetus history, female factors (primary amenorrhea).

In this study, we aimed to demonstrate karyotypes evaluation of the women who were referred to our cytogenetics laboratory.

**MATERIAL- METHOD:** Chromosome preparations were made from 72-h PHA-stimulated cell cultures from peripheral blood. Cell division was synchronized by a pulse of thymidine administered 21 h prior to harvesting. The thymidine block was released by washing the cells in fresh culture medium (RPMI 1640), also prewarmed to 37°C, for 3,5 h prior to harvesting. The culture was harvested using colcemid for 50 min prior to harvesting. At least 20 GTG-banded (Giemsma stain) metaphase cells were analyzed for each patient.

**RESULTS:**

Table 1

Indications	Total	Normal	
RIF	360	342	Mosaic X (n=15) inv(9) (n=7) inv(10) (n=1) balanced translocation (n=4)
RPL	308	282	Translocation carrier (n=10) Inversion (n=6) Mosaic X (n=8) mar (n=1)
Male Factor	44	43	Mosaic X (n=1)
Abnormal Fetus Story	24	23	Balanced translocation (n=1)
Female Factor	18	18	

Table 2

PGD Candidates' Indications(n=261)	Total	Normal Karyotype	
AMA	60	56	Mosaic X (n=4)
RPL	24	23	Mosaic X (n=1)
RIF	82	77	Mosaic X (n=5)
Male Factor	27	27	
Translocation Carrier	43	6	Mosaic X(n=2) Translocation carriers (n=37)

**DISCUSSION:** This study provides the importance of chromosome analysis for female patients applied to IVF center who had the infertility indications mentioned above. According to results, patients who were resulted as translocation carriers were suggested to undergo PGD.

**P02.217****Case report: a balanced translocation between chromosome X and 13**

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We report a 1 year and 9 months patient who was referred to the Tomsk Institute of Medical Genetics with the diagnosis of multiple congenital malformations and neuropsychic development delay. She had microcephaly, deformation and asymmetry of the skull, absence of the part of the left parietal bone. Flat, wide and protuberant bridge of nose («the Greek profile»), hypertelorism, epicanthus and bilateral cleft palate were observed. Palpebral fissures of the various size were located asymmetrically. The philtrum was short and exposed the upper incisors that were set in a “rabbitlike” forward slant. Deep neurologic and behavioral development delay and epilepsy were noted. Standard cytogenetic analysis have revealed a balanced rearrangement: 46,X,der(X)(pter→Xcen),t(X;13)(Xqter→Xq12::13cen→13qter), -13. Dual-colour FISH with DXZ1 and D13Z1/D21Z1 probes allowed to refine localization of centromeres in chromosomes involved. As a result two derivatives were described. The first was presented by der(X) with short arm of X-chromosomes and centromeric heterochromatin. The second one was a consequence of translocation between long arms of chromosomes X and 13. The influence of both derivatives to phenotype can be realized through two mechanisms, including manifestation of X-linked recessive mutations under releasing of X-inactivation and spreading of X-inactivation to autosomal segment with subsequent functional monosity. Our patient has not revealed any symptoms of X-linked disease but clinical features of partial monosity 13. This finding allows to suggest X-inactivation spreading mechanism that is under investigation.

**P02.218****A report of a XYY man with mental retardation, prognathism, and short stature**

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Here we report a 24-year-old man referred to our laboratory for evaluation of possible Fragile X syndrome on the basis of mental retardation. The patient was found to be negative for Fragile X. But he was found to be 47 XYY from chromosomal examinations. On clinical examination the patient was found to have dysmorphic facial features, prominent ears, long fingers, short stature, crowded teeth, high arched palate, prognathism, low nasal bridge, large testes, and speech problem.

We have reported previously another case of XYY male with mild mental retardation, prognathism and malformation of nails and hands. The common features of this new case with the previous one are mental retardation and prognathism.

**P02.219****Prevalence of gr/gr deletion among Iranian azoospermic infertile men**

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Number of microdeletion in the AZF locus of Y chromosome which itself is divided in to three discrete regions (AZFa, AZFb and AZFc) has been demonstrated to play important role in impaired spermatogenesis. The most frequent deletions are known to be occurred in AZFc

region of AZF locus and different prevalence of the partial deletions were reported in different populations.

In present study, we have screened 50 nonobstructive azoospermia men and 50 fertile men for gr/gr deletion of DAZ gene. Tow AZFc-specific sequence-tagged sites were examined using multiplex PCR method. The prevalence of gr/gr deletions in patients and controls samples were 32% (16/50 cases) and 6% (3/50 cases) respectively. The difference between two groups was significant (p-value: 0.011) Our results suggest that gr/gr partial deletion is more frequent in infertile men and may be correlated to the Iranian male infertility.

#### P02.220

#### Y chromosome microdeletion screening in infertile men in Serbia

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The purpose of this study was to evaluate frequency Y chromosome microdeletions in Serbian men with azoospermia and severe oligozoospermia. Initially, 206 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 or known cytogenetic defects were present. The screening for Y chromosome microdeletion was performed in 176 selected patients by multiplex polymerase chain reaction (PCR) method on DNA extracted from peripheral blood. The following STS markers were tested: sY 84, sY 86 (AZFa); sY 127, sY134 (AZFb); sY 254, sY 255 (AZFc). Amplification found to be negative were repeated at least two times to confirm the deletion of given marker. The presence of deletions was correlated with patient's sperm concentration, and hormonal parameters. In 6,1 % men with sperm concentration less than  $1 \times 10^6$ /ml a Y chromosome microdeletions were detected, comparing to 8,5 % in azoospermic men. The most frequent deletion was AZFc. Conclusion: The clinical correlation of spermatogenic impairment to the different AZF region deletions may provide the useful information for genetic counseling of infertile couples.

#### P02.221

#### Genetic studies in 50 patients with Functional Azoospermia

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The present study investigates the number of molecular and cytogenetic defects in a population of 50 azoospermic men, (age 33.36 range 26 to 50) from the Western Region of Saudi Arabia from January 2006 to December 2007. Functional azoospermia was defined as total absence of spermatozoa in ejaculate, with normal vas deferens and without obstruction.

Cytogenetic analysis was performed in peripheral blood culture. GTG-banding of 20 metaphases was done. FISH with X centromeric (CEP X), Y centromeric (CEPY), SRY LSI probes was used. In all cases of mosaicism 500 cells were scored by FISH.

Y microdeletions were detected by multiplex PCR according to the European Molecular Genetics Quality Network guidelines for Yq deletion testing, using gene-specific primers that covered all three regions of the azoospermic factor (AZFa, AZFb and AZFc). This included sY84, sY86 for AZFa; sY127, sY134, for AZFb; sY254, sY255 for AZFc.

Chromosome abnormalities were observed in 16 cases (32%), including 47,XXY karyotype in 11 cases, three mosaic (one 47,XXY/46,XY/45, one 47,XXY/46,XY and one 46,XY/45,X). One patient was 46,XX male, SRY gene was detected by FISH. One patient had a terminal deletion of chromosome 19p13. Y microdeletions were found in three cases (6%): two with AZFc, and one with both AZFc and AZFb. Genetic abnormalities were determined in 38% of azoospermic cases, which is higher than the usual reported figures between 15% to 30%. Genetic testing is indicated to determine etiology of azoospermia and to choose ART strategies and the offering of prenatal diagnosis in the indicated cases.

#### P02.222

#### An azoospermic man with a rare sex chromosome mosaicism

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Among numerous etiologic factors, chromosomal aberrations play a prime role in male infertility accompanied by abnormal semen. The patients with most severely compromised sperm counts have sex chromosome aberrations including aneuploidy and the presence of marker chromosomes. Some patients have evidence of mosaicism as detected in their peripheral blood karyotypes. Here, we report a 28-year-old patient with a rare sex chromosome mosaicism. His chief complaint was infertility secondary to azoospermia. Chromosome investigations were performed on cultures of peripheral blood lymphocytes using GTG-banding and HR-banding techniques. His karyotype showed: 47,XXY / 47,XXX / 48,XXYY. The origin of the error that leads to this mosaicism is likely to be paternal nondisjunction at meiosis I in one cell lineage, at meiosis II in the second line, and at both meiosis I and meiosis II in the third cell lineage. This was the only case with this kind of mosaicism observed during the last 20-years of work in our Department, and no similar cases were found in the literature.

#### P02.223

#### 20-year chromosomal studies among the Iranian infertile men

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Chromosomal aberrations have been postulated to be as one of the principal genetic factors in male infertility and occur in about 2-3 % of unselected patients with proven subfertility. This rate is estimated to be 5-7 % in patients with oligozoospermia and increasing to 10-15 % in patients with azoospermia. In this 20-year retrospective study we investigated 829 men which were referred to our Department due to infertility. Karyotyping was performed on peripheral blood lymphocytes according to standard methods. Out of 829 patients, 557 patients (67.19 %) had a normal karyotype and 272 patients (32.81 %) showed abnormal chromosomes. Klinefelter syndrome, found in 195 patients (23.52 %), was the most frequent aberration in our study. The remaining 77 cases ( 9.29 %) showed the following abnormal karyotypes:

Karyotype	Number
47,XXY	195
48,XXYY	4
mos48,XXYY/47,XXY/47,XYY	1
mos48,XXXYY/47,XXY/46,XY	4
mos47,XXY/46,XY	9
mos45,X/46,XY	7
mos47,XXY/46,XX/46,XY	7
mos46,XX/46,XY	5
mos45,X/46,X,del(Y)(q10)	2
mos47,XXY/45,X/46,XY	4
mos46,XY,t(7;13)/46,XY	1
47,X,i(X)(q10)Y	3
46,X,add(Y)(p11.3)	7
46,X,del(Y)(q11)	6
46,X,r(Y)	2
47,X,t(X;Y)(q10 q10)	2
46,X,t(Y;14)	2
46,XY,t(14;22)	1
47,XYY	2
46,XX (sex reversal)	5
46,XY,inv(9)	3
Total	272

#### P02.224

#### Analysis of heteroploidy frequency in sperm from man with chromosome rearrangements

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Structural chromosome aberrations detected on metaphase plates from peripheral blood lymphocytes lead to increased risk of chromosome abnormal progeny. Analysis of chromosome segregation in sper-

matozoa from carriers of chromosome aberrations allows to understand peculiarities of meiotic divisions and get information about risk of conceiving chromosome imbalanced offspring.

FISH with specific DNA probes heteroploidy frequency was scored in decondensed sperm from 3 patients with chromosome aberrations: 46,XY,t(2;3)(q33;q29) - case 1; 46,XY,inv(4)(p12q21),inv(10)(p11q21) - case 2 and 45,XY,der(13;14)(q10;q10) - case 3.

In case 1 disomy frequency of chromosome involved in this rearrangement increased (up to 3,01%). In case 2 disomy frequency of inverted chromosome (0,9%) was compared to this one of other autosomes (1,06% for chromosome 7; 0,9% for chromosome 9). Frequency of diploidy was higher in patients with robertsonian (case 3) and reciprocal translocations (case1) (1,48% and 2,05%, respectively) compared to patient with inversions (0,54%). Frequency of heteroploidy are in agreement with spermological analysis (WHO standard): patients 46,XY,t(2;3)(q33;q29) and 45,XY,der(13;14)(q10;q10) feature oligoastenozoospermia, in contrast to patient 46,XY,inv(4)(p12q21),inv(10)(p11q21) having astenospermia.

Our data suggest that spermological analysis can be linked to the karyotype abberations and carriers of chromosome aberrations with decrease of sperm concentration have poor fertility prognosis.

Supported by CRDF and RFBR.

#### P02.225

#### A Y chromosome with three short arms in a male with a 45,X/46,X,psu dic(Y) karyotype

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We report a case with an unusual pseudo-dicentric Y chromosome with three short arm segments. When the male patient was first referred for karyotyping because of hypogonadism and mental retardation more than 30 years ago, he was found to have a mosaic karyotype with two cell lines, one 45,X and the other with an abnormal non-fluorescent Y chromosome. The father had a normal Y chromosome. At the age of 47, the patient was referred again for cytogenetic analysis because of his mental retardation. Mutation analysis in connection with thyroid dysfunction revealed a thyroid hormone receptor beta (TRB) germline mutation. FISH studies revealed a complex rearrangement with an alternating pattern of Y short and long arm material: short-long-short-long-short. Using probes for the subtelomeric regions two signals with the Ypter probe was found whereas no signal was observed with the subtelomeric Yq probe. Two signals were also found using probes for the centromeric region and the SHOX gene. In contrast, FISH with a probe for the SRY gene revealed three signals on the abnormal Y chromosome: two on the short arms of the dicentric and an extra signal in the short arm segment situated in the middle of the rearranged Y chromosome. DNA-studies are in progress to further elucidate the nature of this complex Y chromosome rearrangement.

#### P02.226

#### Cytogenetic Analysis of Infertile Iranian men

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Male infertility factor accounts for about half the cases of couple infertility.

Some of the chromosomal changes (aberrations) which seem effective in men infertility include:

1. Balanced chromosomal translocation
2. Chromosome inversion
3. Marker chromosome
4. Sex chromosome abnormality

Our investigation provides the circumstantial and direct evidences which confirm the importance of the sex chromosome in reproductive disorders. We have analyzed (7years study) 845 blood samples from infertile men which 617 of them were oligospermic and azoospermic. Constitutional chromosome aberrations were diagnosed in 278 of these patients. We have observed 29.2% chromosomal abnormality in azoospermia men that is compatible with the data from literature.

The following abnormal chromosome complements were found:  
46,XX;47XXY;47,XYY;48,XXX;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 have found some patients with complex structural and aneuploidy abnormalities:

× 46,XX,inv(9)(p<sub>11..q<sub>13</sub></sub>)/47,XXY,inv(9)(p<sub>11..q<sub>13</sub></sub>)[4]

× 47,XXY[93]/48,XXY+mar[4]/48,XXX[2]

× 47,XXY,inv(9)(p<sub>11..q<sub>13</sub></sub>)

× 47,XXY,t(1;17)(p<sub>36..1..q<sub>21</sub></sub>)

× 46,X,del(Y)(q<sub>11..2</sub>)[98]/45,X[6]

× 47,XXY,inv(9)(p<sub>11..q<sub>13</sub></sub>)/t(10;22)(q<sub>26..3..q<sub>13..1</sub></sub>)

× 46,X,idic(Y)(p<sub>11..32..q<sub>11..32</sub></sub>)[27]/45,X[36]/46,XY[2]

We believe that many infertilities especially severe oligospermic and azoospermic cases raise the need for a cytogenetic analysis besides molecular techniques to reveal the existence of any genetic abnormalities.

#### P02.227

#### Meiotic studies in spermatocytes from fertile males

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**BACKGROUND:** Meiotic chromosome abnormalities are associated with male infertility. However, little is known about meiotic process in men of proven fertility. We carried out meiotic studies in spermatocytes to establish the base line level of meiotic abnormalities in fertile males.

**MATERIAL AND METHODS:** Testicular biopsies were obtained from 11 fertile males aged 30-47. Meiotic spreads were prepared by the air-drying technique and cells were stained with DAPI. **RESULTS:** Meiotic progression was analysed at pachytene, metaphase I and metaphase II stages, counting 1,000 pachytene spermatocytes per individual. All males showed a normal meiotic progression, with a proportion of metaphase II to metaphase I higher than 0.5. A total of 848 spermatocytes at metaphase I was studied. The overall percentage of dissociated sex chromosomes was 21.7%. Autosomal synaptic abnormalities were found in 0.9% of spermatocytes I (medium-sized bivalents with one chiasmata or small bivalents as two separated univalents). Only 0.2% of the spermatocytes I analysed were hyperploid. Hypoploidy was not evaluated, since it could be due to technical artefacts. The percentage of structural chromosome aberrations per individual ranged from 0% to 5.8%. The total mean chiasma frequency was 51.0, ranging from 48.7 to 53.0. **CONCLUSION:** The incidence of meiotic abnormalities (both chromosomal and synaptic abnormalities), as well as spermatocyte distribution and chiasma count in fertile males could be used as reference data in further studies on males with idiopathic infertility.

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#### P02.228

#### Increased autosomal structural aberrations in human spermatozoa regarding age

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**Background:** Although most of de novo structural aberrations are paternal in origin, little information is available on the relationship between advanced paternal age and structural chromosome abnormalities in human spermatozoa. **Objective:** To explore the age effect on the frequency of structural abnormalities in human spermatozoa. **Methods:** Fluorescence *in situ* hybridisation (FISH) with a panel of 62 specific probes was used in spermatozoa to screen all 22 autosomes for duplications and deletions. Sperm samples were collected from 10 healthy males: five males aged 24-37 (mean age 27.8 years) and five males aged 60-74 (mean age 66.4 years). A total of 150,000 sperm nuclei were scored by multicolour FISH, analysing 1,000 spermatozoa per donor and per autosome. **Results:** The frequency of structural abnormalities per individual ranged from 4.2% to 7.8%. The mean percentage of autosomal duplications was significantly greater ( $P < .05$ ) in older donors (4.5%) when compared with that in younger donors

(3.3%). This increased frequency of duplications in older donors could correspond, at least in part, to an excess of acentric fragments in their spermatozoa. Chromosome 12 was the only autosome with an increased level ( $P < .05$ ) of structural aberrations in relation to age.

**Conclusions:** Our results indicate an association between advanced paternal age and increased risk in offspring for *de novo* autosomal unbalanced structural aberrations.

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#### P02.229

#### A two chromosome inversions 46,XY,inv(4)(p12q21),inv(10)(p11q21) associated with spermatogenetic failures

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Chromosome aberrations carriers are often affected by reproductive failures, including spontaneous abortion and newborns with chromosome abnormalities, due to production of genetically unbalanced gametes. But such patients do not exhibit any particular phenotypes. A man from infertile couple was studied to determine the karyotype and spermatogenesis failures.

Using QFH-banding technique and FISH with DNA probes specific to chromosome 4 and 10, we detected karyotype with two pericentric inversions: 46,XY,inv(4)(p12q21),inv(10)(p11q21). There were no chromosome anomalies in the karyotype of his twin brother (46,XY), their parents were not available for genetic analysis.

Semen parameters (concentration, motility and morphology) were analyzed according to WHO criteria. Motile spermatozoa amounted to 25% with other parameters being normal (asthenozoospermia). Also Quantitative Karyological Analysis of Immature Sex Cells (QKAISK) in ejaculate was performed to determine the stage of spermatogenesis block. Partial block of spermatogenesis after MI division and delay of karyokinesis was detected. Frequency of diploid and disomic spermatozoa was identified using multicolour FISH with specific DNA probes to chromosomes 9 and 10. Frequency of aberrant chromosome disomy did not differ from that of normal one and totaled 0,9%. Frequency of diploid spermatozoa was 0,54%.

This study confirms the necessity of complex cytogenetic somatic cells and gametes analysis for patients with chromosome aberrations to calculate the risk of genetically unbalanced offspring more precisely.

Supported by CRDF and RFBR.

#### P02.230

#### Correlation of Antioxidant Enzyme level with Mitochondrial Mutations in North Indian Infertile Men

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Oxidative damage to spermatozoa is a well known potential cause of infertility. The balance between antioxidants and pro-oxidants is essential. Optimum levels of free radicals (Reactive Oxygen Species) are important physiological mediators in normal sperm functioning. Antioxidants as Glutathione Peroxides, Glutathione Reductase (prostatic origin), Superoxide Dismutase (prostatic and epididymal origin) and Catalase (multiglandular origin) prevent oxidative stress (OS). Free radicals induce mitochondrial DNA damage especially in genes regulating Oxidative Phosphorylation (OXPHOS) and result in impaired sperm function and spermatogenesis.

In an ongoing study the correlation between the enzymatic antioxidants, malonaldehyde (product of lipid peroxidation) in the seminal plasma and in blood serum was done, the results were further correlated with semen parameters and mitochondrial DNA integrity. Results showed significant correlation between SOD in blood serum and sperm concentration ( $r=0.699$ , and  $p= 0.008$ ). Positive correlation was observed between the seminal SOD, sperm count and progressive motility. These results are in accordance with several previous studies. No correlations could be established between the seminal MDA, blood serum MDA and semen parameters. In these cases a significant high number of mitochondrial mutations in the OXPHOS genes were observed. This may explain for low sperm count and motility defects in our patients.

The analysis of antioxidant levels in seminal plasma and blood and

their impact on the semen parameters corroborated with mitochondrial mutations and DNA integrity would help to provide a deep insight into the understanding of the OS induced infertility and thus in better management of such cases.

#### P02.231

#### CFTR gene mutations and TT-polymorphism in infertile men of Russia

T. M. Sorokina, L. F. Kurilo, S. A. Kazakova, A. A. Stepanova, V. B. Chernykh; Research Centre for Medical Genetics of RAMS, Moscow, Russian Federation. Cystic fibrosis conductance transmembrane regulator (CFTR) gene mutations are common genetic cause of male infertility. Cystic fibrosis, congenital bilateral and unilateral absence of the vas deferens (CAVD and CUAVD syndromes) lead to azoospermia or severe oligozoospermia. Also CFTR mutations are associated with reduced sperm quality in men who do not present CF phenotype.

We investigated a cohort of 568 men from Russian infertile couples. Fourteen common CFTR mutations (del21kb, ΔF508, ΔI507, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT, L138ins, G542X, W1282X, N1303K, R334W, 3849+10kbC>T) and TT-polymorphism in the intron 8 (IVS8T) have been analyzed.

CFTR mutations were found in 25 out of 568 (4.4%) patients. Following mutations have been revealed: ΔF508 (n=9), del21kb (n=5), W1282X (n=5), 2143delT (n=2), 2184insA (n=1), G542X (n=1), R334W (n=1), 1677delTA (n=1). The 5T allele frequency was 10.6% in examined patients. This allelic variant has been revealed in the heterozygous (n=52 cases), in the homozygous (n=3 cases) and in the compound heterozygote state with common CFTR mutation (n=5 cases). The combined frequency of CFTR mutations and 5T allele was 7.6% in infertile men cohort.

All patients with CFTR mut/5T and 5T/5T genotypes had a diagnosis of azoospermia or severe oligozoospermia. Two azoospermic men with CFTR mutations had extragenital features of cystic fibrosis (chronic bronchitis or a chronic pancreatitis). In one severe oligozoospermic patient with CFTR mutation the CUAVD syndrome associated with renal abnormalities has been diagnosed.

#### P02.232

#### Modelling reduced male fertility in mice

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Human fertility is highly variable and a substantial part of that variation is genetic. The phenotype is hard to measure and involves input from two individuals making whole genome association studies difficult if not impossible to design robustly. Candidate gene approaches based on a knowledge of the underlying processes of gametogenesis have had only limited success. This is a reflection both of the large number of genes thought to be required, particularly for sperm production, and the likelihood that the majority of mutations are recessive requiring rare homozygosity for a mutation at any one locus to produce a phenotype.

Recognising this we have asked if compound heterozygosity at genes encoding proteins required for meiosis can be a factor. SYCE1 and SYCE2 are proteins of the meiotic Synaptonemal Complex. We have shown that SYCE2 is essential for male and female fertility in mice and here we show that this is also true for SYCE1. Heterozygous null animals are fertile in the case of both genes. Mice heterozygous for both null alleles have a ten fold elevation in XY asynapsis and also an increase in unpaired autosomes. Lack of pairing is predicted to result in cell death and a consequent reduction in sperm count. We observe a reduction in sperm count of up to 40% showing that compound hemizygosity in genes involved in the same pathway can have a significant impact.

#### P02.233

#### Gene expression profiling of normal and pathological testis by microarray analysis

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Microdeletions of the AZFa, AZFb and AZFc loci on Yq are detected in about 10% of infertile males. Despite the large amount of data collected in the last years, the biological mechanisms leading to the disruption

of spermatogenesis in Yq deleted patients are still largely unknown. In this study we analyzed by microarray technology the testis expression profiles of patients with idiopathic infertility, patients carries of AZFc deletion and controls (patients with obstructive azoospermia), in order to obtain useful information about the specific genes involved in each different pathological condition. Using the hierarchical clustering average method in 27 microarray experiments we identified in AZFc deleted patients two main gene clusters showing different expression patterns as compared to normal controls and patients with idiopathic infertility. Analysis of these gene clusters using IPA software revealed an interesting down-regulated gene network directly related with spermatogenesis, with the most significant network centred around YBX2 gene (Y box binding protein 2), involved in RNA storage during gametogenesis. This alteration is responsible for the downregulation of the protamine1 and 2 genes evidenced in the same nethwork analysis. In the expression profiles comparative analysis between controls and AZFc deleted patients we also observed an interesting downregulation of the CREM pathway, which is a master controller gene for spermatogenesis. This suggests that impairment of RNA storage and dysregulation of the CREM pathway could represent two of the biological mechanisms underlying spermatogenesis failure in patients with Yq microdeletion.

#### P02.234

#### Identification candidate genes and proteins involved in male infertility through proteomic analysis of human sperm proteins

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Proteomics offers at present the opportunity to compare the relative abundance of the different proteins present in patients and controls with the potential to identify diagnostic markers and candidate proteins to be used in genetic association studies. Towards this goal the proteins present in the sperm cells from sperm samples from infertile patients and from semen donors were analyzed by 2D gel electrophoresis. In each of the 2D maps the intensity of 101 spots previously identified by MALDI-TOF analysis was measured. Additionally, the protamine content and DNA integrity were also determined in each independent sample. Several interesting proteins such as transcription factors, prohibitin, heat shock and proteasome proteins have been identified. Of relevance the expression of an important number of proteins (55 different 2D spots) correlated in independent sperm samples at high statistical significance. Some of the proteins have been found to correlate with DNA integrity and protamine content in infertile patients. Therefore this is proving to be a useful approach leading to the identification of candidate proteins and therefore candidate genes which may harbour mutations or polymorphisms involved in the pathogenic mechanisms present in infertile patients. Supported by BMC006-03479.

#### P02.235

#### Primary male infertility in Izmir / Turkey: a cytogenetic and molecular study of 187 infertile Turkish patients

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Infertility is an important health problem affecting 10-15% of couples. The contribution of male factors to patients is about 30-50%. The main genetic factors in male infertility are somatic chromosomal abnormalities and Y chromosomal microdeletions within Yq11 region. The genes which control spermatogenesis are located in Yq11 region and are known as azoospermia factor genes (AZF). The AZF region has 3 non-overlapping loci-AZFa, AZFb, AZFc which are required for normal spermatogenesis. All these genes are expressed in testicular tissue showing that they play roles in human spermatogenesis. Here, we aimed to detect somatic cytogenetic abnormalities and AZF microdeletions in a sample of 187 Turkish infertile men and to evaluate the frequencies and the characteristics of our primary male infertility data in order to perform appropriate genetic counseling.

Cytogenetic study revealed chromosomal abnormality in 9 subjects (4.8%). In remaining 178 subjects, 7 subjects (3.93%) were detected

to have Y chromosome microdeletions. The AZFc region was the most frequently involved region in affected subjects. One of these subjects showed microdeletion in all 3 regions(AZFa, AZFb, AZFc) and one subject had microdeletions in both AZFb and AZFc regions. Other 5 subjects had microdeletions in only AZFc region. All subjects having microdeletion were azoospermic.

In conclusion, cytogenetic and molecular study should be performed to obtain reliable genetic information for the genetic counseling of primary infertile man. Y chromosome microdeletion diagnosis is useful in decision making for assisted reproductive techniques because the association between some deletions and residual spermatogenesis capacity has been proven.

#### P02.236

#### Oxidative stress and mitochondrial DNA mutations in Oligoasthenoteratozoospermic patients

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Excess production of reactive oxygen species (ROS) establishes oxidative stress (OS) in the semen. Mitochondria are suspected to be the source and target of ROS where mutation in mitochondria can impair the formation/function of mature spermatozoa. So the present study was aimed to correlate the oxidative stress and mtDNA mutation with the sperm parameters of infertile patients. Study includes 62 infertile subjects and 30 fertile controls attending AIIMS, New Delhi, India. Complete semen analysis was performed according to WHO criteria (1999). OS was evaluated by malonaldehyde estimation and sperm mitochondrial DNA mutations by standard PCR-DNA sequencing. Infertile group showed significantly ( $p < 0.001$ ) higher MDA levels ( $0.18 \pm 0.03$  nmol /  $10^6$  spermatozoa) compared to fertile controls ( $0.10 \pm 0.02$  nmol /  $10^6$  spermatozoa). The mean sperm count of infertile group was  $12.78 \pm 11.81 \times 10^6$ /ml, whereas the fertile group had  $55.73 \pm 20.57 \times 10^6$ /ml. DNA sequencing revealed that 66% (n=41) of the infertile group harboured one or more mutations (T4672A, C10165T, C10207T, A10470G, T13946A) in the mitochondrial genome where no mutations were detected in the fertile group inspite some common nucleotide changes (A750G, A4769G) in both the groups. All the mean sperm parameters of infertile subjects were negatively correlated with the malonaldehyde level. Higher MDA levels in the infertile subjects compared to the controls may be correlated with the change in the gene sequence of sperm mtDNA of infertile subjects. Thus mtDNA mutation harboured in the infertile groups may be due to oxidative stress.

#### P02.237

#### Mutations and polymorphisms in the cystic fibrosis gene in men with severe oligozoospermia.

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Background: Majority of male cystic fibrosis (CF) patients are infertile because of congenital bilateral absence of vas deferens (CBAVD). In addition, male infertility as a result of isolated CBAVD is also recognized as a primary genital form of CF. Mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene have been found in more than 85% of CBAVD cases. Possible involvements of CFTR mutations in other forms of male infertility have been suggested but remain controversial. In sperm, CFTR may be important in transport of various anions and sperm capacitation, hence making it possible that certain CFTR mutations may lead to reduced sperm fertilizing capacity and male infertility in other forms of male infertility rather than CBAVD.

Objective: To compare the frequency of CFTR gene mutations between oligozoospermic and healthy fertile men.

Materials and Methods: The study populations consisted of 124 oligo-

and 90 normozoospermic men. We screened simultaneously for 254 different CFTR mutations and variations using arrayed primer extension (APEX) genotyping microarray (Asper Biotech Ltd). Results: CFTR mutations and variants were demonstrated in 22 (17.7%) of 124 oligozoospermic patients and in 13 (14.4%) of 90 control men. In addition, the total frequency of mutant/variant alleles in infertility group was slightly, but not significantly higher than in controls (9.7 vs 7.2%). Similar trend was also observed for IVS8-5T allele frequencies (3.6 vs 2.2%, respectively). Although we demonstrate comparable CFTR mutation/variation frequencies in both groups, the causal relationships between specific CFTR mutations and male infertility cannot be completely ruled out.

#### P02.238

#### Simultaneous inactivation of the transcription factors Sox9 and Sox8 in murine testis development leads to complete infertility

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Heterozygous loss-of-function mutations of the HMG-box transcription factor SOX9 result in campomelic dysplasia, a human skeletal malformation syndrome associated with XY sex reversal. The murine Sox9 gene is expressed in embryonic and postnatal Sertoli cells of the mouse testis, and inactivation of Sox9 before the sex determination stage at E11.5 leads to complete XY sex reversal. To see whether Sox9 is required for testis development after testis induction, we crossed Sox9<sup>flox</sup> mice with an AMH(Anti-Müllerian Hormone)-Cre transgenic line. Conditional Sox9 null mutants, SOX9-negative at E14.0, are initially fertile, but become sterile from complete meiotic arrest at around 5 months. As Sox8, a Sox9 related transcription factor, i) is expressed similar to Sox9 during murine testis development, ii) has been shown *in vitro* to activate AMH, a Sox9 target during testis development, iii) is expressed normally in AMH-Cre;Sox9<sup>flox/flox</sup> mutants, and iv) as homozygous Sox8 null mutants show no obvious early gonadal phenotype, we hypothesized that Sox8 may compensate for the absence of Sox9. We therefore generated a Sox9 conditional knockout on a Sox8 mutant background. In double mutants heterozygous for Sox8, testes develop normally up to post-natal day 10 (P10), but subsequently show spermatogenic arrest. Homozygous double mutants show normal testis cord formation at E15.5, but subsequent testis cord development is impaired; at P6, testis cords are completely irregular in shape and appear fibrotic, resulting in complete infertility. In summary, concerted function of Sox9 and Sox8 in post E14.0 Sertoli cells is essential for the maintenance of testicular function.

#### P02.239

#### Real-Time PCR evaluation of TSPY copy number in infertile and seminoma patients.

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Introduction: Multicopy TSPY gene is localized on chromosome Y in MSY region in gene clusters. Total number of the gene copies is estimated from 20 to 40. Testis specific expression indicates a role in meiotic or post meiotic processes during spermatogenesis.

The aims of this work are:

- 1) Confirmation of our previous findings (Vodicka R, et al., TSPY gene copy number as a potential new risk factor for male infertility. Reprod Biomed Online. 2007 May;14(5):579-87.)
- 2) Analyses and comparison of a new DNA samples from infertile and seminoma patients.

Material and method: There were included 104 infertile and 6 seminoma patients and 50 healthy controls into study.

Copy number relative quantification was measured using the combination of two Real -Time PCRs by Y quantifiler kit and by SYBR green kit.

Results: Our results confirmed increasing copy number in infertile patients (in average 53 relative TSPY copies) compare to controls (in average 31 relative copies) even after enlarged collection of samples.

In addition the seminoma patients showed twice as many copy number compare to the infertile patients (in average 102 relative copies).

Conclusion: The main importance of our findings lies in great diagnostic potential in male infertility genetic background testing. Number of TSPY copies could be also significant tumor marker in relation to testicular tumorigenesis.

#### P02.240

#### AZF microdeletions on the Y chromosome of Iranian infertile men with non-obstructive azoospermia

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The human Y chromosome contains genes that are essential for spermatogenesis specially those that are located on three major intervals defined as AZFa, AZFb and AZFc.

Deletions in these genes may result in spermatogenic failure in patients with non-obstructive azoospermia and oligozoospermia. Widely different frequencies of such deletions (0-55%) have been reported from different populations.

The main purpose of this study is to detect the frequency of Y chromosome microdeletions in Iranian patients with non-obstructive azoospermia and fertile control subjects. Multiplex polymerase chain reaction (PCR) was applied using several sequence-tagged site (STS) primer sets, in order to determine Y chromosome microdeletions in 100 infertile males and 50 fertile controls.

Microdeletions in AZFa, AZFb and AZFc (DAZ gene) regions were only detected in seventeen of the patients (17%) with the frequency of 15%, 49%, and 36% respectively.

Our findings suggest that knowing the prevalence of AZF microdeletions in Iranian infertile men will be informative before starting assisted reproductive treatments.

#### P02.241

#### Male infertility and biotransformation enzyme gene polymorphisms

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Environmental xenobiotics such as organophosphate pesticides are known to be involved in male infertility. Interindividual genetic variations in biotransformation enzyme activities can lead to differences in the susceptibility to male infertility. In this case-control study, PCR was used to investigate the association between polymorphisms in the PON and GST genes (PON1-55/192, PON2-311, GSTM1/T1) and male infertility in 381 Slovenian participants (the study group of 187 infertile male participants: 86 with azoospermia, 101 with oligoasthenoteratozoospermia; the control group of 194 fertile males).

We found statistical significant difference in the PON1-55 genotype distribution between the infertile and fertile men ( $\chi^2 = 7.37$ ;  $p = 0.02$ ), which after applying Bonferroni correction was no longer significant. Likewise, no significant differences in frequencies of genotypes of other tested polymorphisms, PON1-192, PON2-311, GSTM1/T1, respectively, and the occurrence of male infertility were observed (Table 1).

In this case-control study we didn't confirm the association between PON1/2 or GSTM1/T1 genetic variations and male infertility in Slovenian participants. However, limitations of the genetic association studies, namely, the relatively small sample size and population specific genotype effects which make results difficult to reproduce, should be considered when interpreting and generalizing the results.

Table 1. Genotype frequencies of the PON and GST polymorphisms in infertile and control group.

Genotype	Infertile group (N = 189)	Control group (N = 194)
PON55 LL	73	97
ML	93	87
MM	21	10
chi-square(2)	7.37	
p	0.02	
PON192 QQ	98	91
QR	74	90
RR	15	13
chi-square(2)	1.84	
p	0.40	
PON311 SS	106	92
SC	72	85
CC	9	17
chi-square(2)	4.40	
p	0.11	
GSTM1 null	97	92
normal	90	102
chi-square(1)	0.59	
p	0.44	
GSTT1 null	35	46
normal	152	148
chi-square(1)	1.14	
p	0.29	

**P02.242****Detection of the most common genetic causes of male infertility by QF-PCR analysis**

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The most common genetic causes of spermatogenic failure are sex chromosomal abnormalities (most importantly Klinefelter's syndrome) and deletions of the azoospermia factor (AZF) regions (AZFa, AZFb, AZFc) of the Y chromosome. Recently, several studies have proposed that partial AZFc deletions/duplications may represent a risk factor for spermatogenic impairment. Here we describe a multiplex quantitative fluorescent (QF)-PCR method that allows detection of these common genetic causes and risk factors of male infertility. The 11-plex QF-PCR included the amplification of amelogenine gene; four polymorphic X-specific short tandem repeats (STR) markers (XHPRT, DXS6803, DXS981 and exon 1 of the AR gene), non-polymorphic Y-specific marker (SRY gene), polymorphic Y-specific STR marker (DYS448), as well as co-amplification of DAZL/DAZ, MYPT2/MYPT2Y and two CDY1/CDY2 fragments that allowed for the determination of the DAZ, MYPT2Y and CDY gene copy number. A total of 348 DNA samples from infertile/subfertile patients (n=204) and fertile controls (n=144) were included in the study. We detected 14 infertile males with sex chromosomal aneuploidies (10 individuals with Klinefelter's syndrome, two XX and two XYY males). All previously detected AZF deletions; AZFc (n8), AZFb (n1), AZFb+c (n1), gr/gr (n11), gr/gr with b2/b4 duplication (n3) and b2/b3 (n5) gave a specific pattern with the 11-plex QF-PCR. In addition, 29 DNA samples showed pattern consistent with the presence of gr/gr and two with b2/b3 duplication. In conclusion, multiplex QF-PCR is a rapid, simple, reliable and inexpensive method that can be used as a first-step genetic analysis in infertile/subfertile patients.

**P03. Prenatal diagnostics****P03.01****Use of array CGH in prenatal diagnosis**

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**OBJECTIVE:** Microarray based comparative genomic hybridization (aCGH) is relatively widely used in genetic diagnosis of children, but true potential is still unexplored in prenatal diagnosis. The objective of

our study is to evaluate the feasibility of BAC arrays in the analysis of prenatal samples.

**METHODS:** Chorionic villus and amniotic fluid samples are obtained using standard clinical procedures. DNA is extracted and labelled for the aCGH analysis. For samples with limited amounts of DNA, we perform whole genome amplification (WGA). Two types of arrays are used: arrays targeted to constitutional syndromes (Constitutional Chip® 3.0), and 1 Mb resolution-arrays covering whole genome (Spectral Chip™). The arrays are scanned with ScanArray®, and data is analysed with SpectralWare®. All samples are also analysed by conventional karyotyping. Potential aCGH findings are confirmed with FISH using the corresponding BAC DNA as probe.

**RESULTS:** To reduce the turn-around time, DNA is extracted directly from the samples. For direct amplification, we have compared three suppliers for their WGA performance. In addition, some cases have been tested both with native and amplified DNA to address potential bias caused by amplification. Results are promising, and a larger set of samples will confirm the best practices.

**CONCLUSION:** The use of samples without cell culturing combined with simultaneous detection numerous genomic imbalances can have great benefit to prenatal diagnosis. The difficulty in discriminating the pathologic and benign copy-number variations and the possibility to detect potentially unwanted information will undoubtedly be challenging for the professionals interpreting the data but also for array manufacturers.

**P03.02****Sequential and Integrated prenatal screening for Down syndrome**

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**Objective.** To compare the integrated test in three variants screening for prenatal Down syndrome detection: the first trimester combined screening , sequential screening and integrated screening .

**Methods.** Ultrasound scanners Aloka SSD-2000 and Medison SA-8000 Live was applied for 716 singleton pregnancies on 10-13 weeks of gestation with 265 of them (37%) were after 35 . First trimester biochemical markers were studied on 9 to 13 weeks of gestation with Life Cycle system for prenatal screening (Wallac/Perkin Elmer Life and Analytical Sciences, Finland). 644 second trimester samples ( 89,9%) were tested by test- systems products of "Alkor-Bio", Saint-Petersburg . Detection rates (DR) and false-positive rates (FPR) were estimated.

**Results.** Integrated screening has the lowest FPR. All 27 cases of chromosomal anomalies (including 22 DS cases) were revealed after prenatal diagnostics with FPR value 13.6% (1st trimester). FPR in the second trimester screening was 23.4%. FPR for integrated screening in this group was twice lower - 11.3%. For young pregnant women FPR was 2.9% comparing to 5.8% FPR after combining screening.

**Conclusion.** Integrated screening was the safest policy in the high risk pregnancies. Interpreting the second test but ignoring the first-trimester markers measurements leads to false risk estimation.

**P03.03****An assessment of the use of interphase FISH in prenatal diagnosis**

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Interphase fluorescence *in situ* hybridization (FISH) with different DNA-probes has been provided more ability to perform chromosomal enumeration. In our study FISH with AneuVysis kit (Vysis, Abbott) was applied for detecting of more commonly aneuploidies in 67 (8,6%) from 779 cases of prenatal diagnosis after CVS. Interphase FISH analysis was carried out when absence or small amount of metaphases took place in semi-direct samples to avoid of repeated invasive procedure. Different aneuploidies were diagnosed correctly and rapidly by FISH in 14 fetuses with phenotypic abnormalities on ultrasound. In other 53 cases fetal aneuploidies were not detected by FISH analysis. In 49 of them fetuses did not have any ultrasound abnormalities and appeared normal at birth. Normal fetal karyotypes were confirmed by conventional cytogenetic analysis on cord blood lymphocytes in 2 cases with abnormal ultrasound screening (intrauterine growth retardation). There was spontaneous termination of pregnancy at 18-19 weeks gestation in one case with fetal cystic hygroma. In remainder case with

abnormal nuchal translucency (7mm) cordocentesis was performed and 46,XX,r(13)(p11q22) karyotype was detected by conventional cytogenetics. Obtained date suggest that FISH may be a satisfactory alternative test in women undergoing prenatal diagnosis because of advanced maternal age, abnormal maternal serum screening, a previous pregnancy with a trisomy 21 and parental anxiety when high quality ultrasound examination is provided. For the other indications, especially for fetal abnormalities, detected on ultrasound scan, it is necessary to follow FISH with conventional cytogenetics.

#### P03.04

#### Prenatal DNA Detection of Down Syndrome

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It has been shown that fetal cells and circulating cell-free fetal DNA increases in the maternal circulation in women carrying Down syndrome fetus. The current technology in non-invasive screening methods of fetal aneuploidies is focused on detecting Y-chromosomal sequences which is not practical to be used for pregnancies involving female fetuses. Hence, it is vital to develop an assay that is universal for both male and female fetus pregnancies. We attempted the use of superoxide dismutase (SOD-1) gene, which is located at the Down Syndrome Critical Region, to overcome this situation for the prenatal detection of Down syndrome. The prospective of the gene using real-time quantitative polymerase chain reaction was explored. Our results show that the level of SOD-1 sequences is significantly elevated in the third trimester normal pregnancies (mean = 11728 copies/ $\mu$ l) when compared to the second trimester (mean = 5705.6 copies/ $\mu$ l),  $p<0.005$  and non-pregnant normal women (mean = 3580.2 copies/ $\mu$ l),  $p<0.0001$ . Down syndrome pregnancies have the greatest elevation compared to all the three trimesters of normal singleton pregnancies and twin pregnancies,  $p<0.05$ . These data indicate that a quantitative analysis using a gene associated with a disorder could be used in screening for the prenatal diagnosis of fetal aneuploidies regardless of the sex of the fetus.

#### P03.05

#### Chromosome 21-specific STR markers can effectively diagnose fetal Down syndrome: a comparison to traditional karyotyping

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Short tandem repeats (STR) have been proposed to be useful in identifying chromosome aneuploidies. In this study, it was our goal to test the sensitivity and specificity of the combination of 3 chromosome 21-specific STR loci in rapid determination of the number of fetal chromosome 21 prenatally. Six hundred and two amniotic fluid samples from increased risk pregnancies (either with abnormal maternal serum screening results or with advanced maternal age) were analyzed. Specific primers with florescent dye labeled on the 5' ends were applied to PCR-amplify these three STR loci following denaturing polyacrylamide gel electrophoresis in a DNA sequencer. Data revealed that 588 amniotic fluid samples showed two chromosome 21s in the fetal cells. However, two of them with 3 chromosome 21s in the fetal cells. These data were 100% concordant to the fetal karyotyping results from the clinical cytogenetic laboratory. In conclusion, this protocol could provide a very rapid yet accurate detection of fetal Down syndrome, comparing to traditional chromosome karyotyping. It is especially useful for those late second trimester pregnancy women that if amniocentesis is pursued and confirmed fetal Down syndrome by karyotyping yet not able to meet the 24 week deadline for therapeutic abortion. This molecular approach could be their alternative for karyotyping.

#### P03.06

#### A comparison between first and second trimester pregnancy screening for Down Syndrome

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We wanted to ascertain which prenatal screening method should be used in practice to detects more cases of Down syndrome, with a lower false positive rates.

For this purpose, we made a retrospective study among the 570 sec-

ond trimester pregnant women and 104 first trimester pregnant women, which underwent triple/double test screening for Down syndrome. Estimating with Prisca 4.0 software :  $\beta$ -HCG , AFP and free-estriol values, obtained by chemiluminescent assay , it was selected a group of 115 second trimester pregnant women with elevated risk for Down syndrome. Among these women, 88 chose not to have amniocentesis .We analysed the outcome of children born by these women and we found a high rate of "false" positive results - about 20%. Of the 27 women with high risk which accepted amniocentesis, 26 cases presented normal fetal karyotypes and one showed a pseudomosaicism.

In contrast , first trimester screening , which means maternal serum PAPP-A and free-HCG measurements, combined with nuchal translucency thickness , showed a much lower 'false" positive rates , for about 5%.

We concluded that first trimester screening is more effective than triple test screening in early detection of Down syndrome

#### P03.07

#### Implementation of a combined screening of aneuploidies

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Multiple strategies of chromosomal anomalies screening have been developed, aimed to reduce the number of invasive techniques.

The first trimester combined test has been widely considered as one of the most efficient approaches.

Objetive: A retrospective observational study has been carried out in order to evaluate the first year of a multicentric screening program in Asturias.

Material and methods: 3630 pregnant women were screened, from 7 hospitals of Asturias. Maternal serum free B-hCG and PAPP-A concentrations were measured in our reference laboratory (HUCA), in a Delfia Xpress analyzer from Perkin Elmer (PE). Nuchal translucency measurement (NT) was performed at every hospital.

Results from genetic analysis in amniotic fluid as well as the outcome of the delivery were also registered in order to evaluate the efficiency. Results: 3630 women during the 9- 13<sup>+</sup> weeks of pregnancy participated in the screening (mean age 31.1 years, 23% older than 35 years). There were 9 Down syndrome cases. Combined screening detection rate was 66.7% (3.97% false positive); 56 % of the amniocentesis corresponded to pregnant women older than 35 years, but only 29 % were indicated for a positive screening result.

Conclusions: The screening program allowed us to offer a prenatal diagnosis to 77% of our pregnant women population, which is under 35 years old.

The false positive rate decreased from 14.2% (maternal age) to 4% (combined test).

Two main points of this program have to be highlighted: the total control of the pregnancy and the continuous optimization based on the multicenter colaboration

#### P03.08

#### Proteomic screening techniques used in maternal plasma reveal potential biomarkers for Trisomy 21 pregnancies.

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Objective: To use proteomic methods to search for biomarkers for trisomy 21 (T21) in maternal plasma.

Methods: Surface Enhanced Laser Desorption Ionisation Mass Spectrometry (SELDI) was used to compare gestationally aged matched maternal plasma samples from normal and T21 pregnancies to detect differentially expressed proteins optimally <30kDa. Analysis was performed using Q10 cation exchange arrays. Two Dimensional Difference Gel Electrophoresis (2D DiGE) which is optimal at screening for larger molecular weight proteins was performed at three expanded pH ranges.

Results: SELDI analysis demonstrated significant differences across gestational ages in normal pregnancies, and changes in T21 pregnancies. First trimester T21 samples showed a small peak at ~100kDa that was significantly elevated by 1.3 fold ( $p=0.00026$ ). Second trimester

T21 samples showed significant changes, with similarities to the protein profile of normal first trimester pregnancies. Two protein peak masses in the ranges 5-10kDa ( $p=0.028$ ) and 20-25kDa ( $p=0.004$ ) were more than 2 fold different. The 2D DiGE first trimester study revealed two spots in the 5.3-6.5 pH range, increased by 1.4 fold ( $p=0.019$ ) and 1.2 fold ( $p=0.017$ ). Analysis of second trimester samples showed two further significant spots in the 5.3-6.5 pH range and two in the 6-9 pH range ( $p<0.05$ ).

**Conclusions:** Proteomic screening methods have revealed a potential first trimester marker for T21 and have demonstrated changes in the normal maternal plasma proteome related to gestational age which do not occur in T21 pregnancies. These proteins may be useful as biomarkers to improve on current screening for T21, reducing the need for invasive testing.

#### P03.09

##### Fetal sex determination service using real time PCR and pyrophosphorolysis-activated polymerization on free fetal DNA

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Routine genetic prenatal diagnosis is currently based on an invasive sampling procedure, which carries a significant risk (1-2%) of fetal loss. Therefore, the possibility for non-invasive sampling has been pursued for several years particularly following the discovery of free fetal DNA (ffDNA) present in maternal plasma. Two techniques, Real Time PCR, that can detect very low levels of Y chromosomal sequence, and a new and highly specific PCR technique, pyrophosphorolysis-activated polymerisation (PAP), were validated for fetal sex determination using free fetal DNA (ffDNA) as a substrate (Prenat Diagn. 2007 **27**: 932-7). Both Real Time PCR and PAP can be used to detect the very low levels of Y chromosome DNA present in ffDNA during a male gestation and in combination offer more reliable early prenatal sexing. Using these two techniques combined a service for fetal sex determination from maternal blood is now offered in the NW Regional Molecular Genetics Laboratory, St Mary's Hospital, Manchester. In the 8 months that the service has been running 27 patients have been tested (9 male, 11 female and 7 fail). The predicted sex of the fetus was subsequently confirmed as correct in 8 of the 27 samples tested, and we are not aware that any of the remaining 12 results were incorrect. The failure rate is currently 15%, which is partly due to the strict test acceptance criteria we impose on this new service. The test acceptance criteria are that 4/4 PAP replicates and 7/8 Real Time Ct values must be in agreement.

#### P03.10

##### Introduction of MAQ technique for autosomal aneuploidies detection

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Multiplex Amplicon Quantification (MAQ) is a multiplex PCR based method for the detection and analysis of CNVs. The method consists of the simultaneous PCR amplification of several fluorescently labeled target and reference sequences. The comparison of normalized intensities between the test individual and control individuals results in a dosage quotient indicating the copy number of the target amplicon. The Trisomy MAQ for the detection of autosomal aneuploidies contains 42 amplicons divided over 34 test amplicons: 7 on chromosome 21, 7 on chromosome 18, 8 on chromosome 13, 9 on X and 3 on Y chromosomes and 8 control amplicons. The MAQ assay was applied on 615 DNA samples derived from amniotic fluid or chorionic villi of which 535 samples had normal karyotypes and 80 samples had abnormal karyotypes. All investigated samples were previously diagnosed by STR based QF-PCR and cytogenetic analysis.

Genescan was performed on ABI 3700 DNA sequencer (Applied Biosystems) data was analyzed with MAQs software ([www.vibgeneticservicefacility.be/index.htm?soft=maqs.php](http://www.vibgeneticservicefacility.be/index.htm?soft=maqs.php)).

The influence of the input DNA quality, concentration and solvents used for extraction on the efficiency of MAQ was estimated. The optimal concentration was found to be between 24 and 44 ng/ $\mu$ l.

The obtained results confirm that MAQ is highly sensitive technique that requires DNA samples of good purity and minimum concentration. MAQ's experimental setup solely consists of a standard (multiplex) PCR reaction followed by fluorescent fragment analysis, together resulting in a considerable reduction of labor and time.

#### P03.11

##### Reliability of results obtained using subtelomeric MLPA in prenatal samples. Our experience

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MLPA (Multiplex Ligation-dependent Probe Amplification) is a technique which allows to analyze large numbers of samples simultaneously and has proven to be accurate for identifying deletions, duplications, and amplifications in several diseases and for the screening of subtelomeric rearrangements. The aim of our study is the diagnosis and characterization of cryptic subtelomeric chromosomal abnormalities in a series of pregnancies in which ultrasound examination discloses major fetal malformations, using a specific set of probes for subtelomeric chromosomal imbalances, SALSA P036B,D human telomere kit (MRC-Holland). It was performed on fetal DNA obtained from cultured amniocytes. To exclude false positives we use the complementary kit P070 (MRC-Holland). We have collected 173 amniotic fluid samples from pregnancies in which fetuses present different phenotype abnormalities and show a normal karyotype. Investigation revealed 23 cases that presented subtelomeric imbalances. These submicroscopic chromosomal anomalies include deletions and/or duplications of several chromosomes. Tests performed with both kits gave 3 confirmed imbalances (3pter deletion, 13qter duplication plus 20pter deletion) and 21 discordant results. These controversial results may be due to different reasons. Some P036 imbalances have been previously reported as polymorphisms. Another reason is that P036 and P070 kits contain different probes and some imbalances are located very closer to the telomere region so the P070 kit does not confirm the results and FISH is not enough sensible to detect them. These discordant results need more accurate techniques for confirming some subtelomeric imbalances.

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#### P03.12

##### Multi-colour FISH in 17 minutes: Towards 24 chromosome aneuploidy screening in under 24 hours

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Preimplantation genetic screening (PGS) for chromosomal aneuploidy has become widespread for women of advanced maternal age in recent years. The 'gold standard' technique (multicolour fluorescence in situ hybridisation (FISH) to identify up to 12 specific chromosomes after multiple rounds of hybridization) has received criticism owing to the possibility of false positives and the possible omission of key chromosomes not included. Any successful PGS test needs to be quick, simple, cheap and accurate. We describe preliminary results using fast FISH to simultaneously screen multiple chromosomes in lymphocytes as a prelude for clinical application. 5 colour FISH was achieved in only 17 minutes from slide preparation to final post-hybridization wash (including a 5 minute hybridisation time). In the initial experiments, probes for X, Y, 15, 17 and 20 have been successfully applied to control lymphocytes. In a second set of experiments, four hitherto poorly studied chromosomes (1, 8, 11 and 17) in human embryos and sperm were analysed. Finally we have applied 4 sequential layers of probes (14 probes in total) in approximately 7 hours. Despite moves to develop microarray approaches to simultaneously analyse 24 chromosomes conventional FISH analysis should still be useful for single cell diagnosis and will certainly be useful on a per cell basis for any 'abnormal' embryos to confirm the single cell diagnosis.

**P03.13****Inconsistent findings between QF-PCR and karyotype analysis for the prenatal diagnosis of common trisomies in amniocentesis**

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We report an amniocentesis case of discrepant results between QF-PCR and karyotype analysis in the prenatal diagnosis of trisomy18. The result of rapid QF-PCR analysis from uncultured amniotic fluid cells indicated double autosomal aneuploidy for chromosome 18 and 21. However, subsequent cytogenetic analysis from the in situ cultures showed only trisomy 21 karyotype with no evidence of mosaicism. Follow-up studies were performed on the repeated amniocentesis, fetal cord blood and placental tissue after termination of the pregnancy. QF-PCR and karyotype results showed trisomy 21 from all samples. For chromosome 18, both QF-PCR and karyotyping from placental tissue revealed a normal disomic chromosome 18. Cord blood and repeated amniotic fluid cells indicated the aneuploidy of chromosome 18 in QF-PCR, but normal disomy 18 with no evidence of mosaicism in karyotyping. We supposed that this inconsistent finding was the result of duplication of the paternal chromosome 18 in mitosis after the loss of the homologous maternal chromosome 18 at an early postzygotic stage.

**P03.14****QF-PCR on native chorionic villous for false positive and negative detection is a useful tool in fetal karyotyping by direct method**

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The prenatal diagnosis(PD) on chorionic villi (CV) more reliable for the identification of fetal karyotype combines the cytotrophoblast (direct method, STC) and mesenchyme (long term culture, LTC) analyses. However, when the sample available is poor (5-10mg) the diagnosis is generally performed looking better for the direct method in order to avoid the maternal cell contamination. This condition is associated with a higher false negative risk. In our 7 years experience on 24'237 CV prenatal diagnosis, we recognised 18 cases of type V True Fetal Mosaicism (TFM) that, if they have been analysed only with STC they would have been subject to a false negative result. The abnormality found in mesenchyme of 17/18 was an aneuploidy for chromosomes 13,18,21,X,Y. In 11 of them the abnormal cell line was present in a homogeneous form, hence, possibly recognizable by QF-PCR. On these basis we performed a study to calculate in our survey the false positive and negative risk if PD would have been performed only with direct method and to evaluate in 267 poor samples (5-10mg) if QF-PCR on a minor fragment allows detection of false positive and negative cases. We evidenced 1 false negative and 2 false positive cases and in 14 instances the abnormality found in cytotrophoblast was confirmed by QF-PCR. In conclusion, QF-PCR could be an additional useful tool, which doesn't jeopardize the result of direct method, to decrease the false negative risk from 1/1136 to 1/2941.

**P03.15****Rapid prenatal diagnosis of aneuploidies by QF-PCR: evaluation of large scale clinical application**

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Introduction: Recently it has been shown that rapid QF-PCR can detect the great majority of chromosome abnormalities in prenatal samples despite being deliberately targeted to chromosomes 21, 18, 13, X and Y. Main advantages of the assay are low cost, speed and automation

allowing large scale application.

Methods: We developed a QF-PCR assay that has been applied on 38.000 clinical samples. Most frequent indications were increased biochemical risk (32%) and advanced maternal age (30%), 6% of these cases were also associated with increased nuchal translucency; parental anxiety and abnormal ultrasound were present in 22% and 7% of samples respectively. Cytogenetic analysis was performed in all cases and results compared.

Results: All 1278 non mosaic aneuploidies involving chromosomes 21, 18, 13 X and Y were detected with 100% sensitivity and specificity; several cases of mosaicism and partial trisomies were also identified. QF-PCR detected 95% of clinically significant abnormalities diagnosed by cytogenetic analysis in samples referred for abnormal ultrasound. Affected pregnancies could be terminated without further waiting for completion of fetal karyotype

Conclusions: Large scale application of QF-PCR could reduce the load of prenatal cytogenetics if all pregnancies are carefully monitored by non invasive methods. In cases of negative QF-PCR results cytogenetic analyses might only be performed for fetuses with abnormal ultrasound; affected pregnancies identified by QF-PCR can be terminated in a few hours from sampling. In countries where large scale cytogenetics is hampered by its cost and lack of technical expertise QF-PCR may be used as the only prenatal diagnostic test.

**P03.16****Prenatal diagnosis of trisomy 21 by real-time PCR on fetal DNA from amniotic fluid**

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Trisomy 21 is the most common congenital anomaly. A region called Down Syndrome Critical Region (DSCR), extending from DNA marker D21S55 on chromosome 21, is critical for Down syndrome phenotype such as morphological features, hypotonia and mental retardation. Recently, there have been major advances performing in the screening and prenatal diagnosis of Down Syndrome. With the advent of real-time PCR, it is now possible to measure the nucleic acid concentrations with high accuracy. This study was undertaken to establish a rapid prenatal diagnosis of trisomy 21 using real-time PCR of fetal DNA from amniotic fluid. Fetal DNA's from the amniotic fluid of a mother expecting a baby with regular trisomy 21 and 9 mothers at high risk for regular trisomy 21 were isolated. Real-time PCR technique was used to measure the dosage of *DSCR3* gene. The amplification plots of 40 cycles for each sample was determined by the real-time PCR using fetal DNA's from the amniotic fluid.  $C_t$  ("cycle threshold": the PCR cycle number at which a significant increase in the components of the reaction is first detected) values for each sample were obtained and the average  $C_t$  values were calculated. The *DSCR3* and *GAPDH* gene dosage were statistically determined and a comparison was made between the results of the risk group and the one with regular trisomy 21. The real-time PCR results showed the *DSCR3* gene dosage of one of 9 samples from the risk group was higher than the one with regular trisomy 21.

**P03.17****Introducing new methods in prenatal diagnosis can do harm more than good. The need for a national strategy**

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Screening programs (SP) is indispensable part of health care systems in many countries. During the past 50 years new SP are becoming available. The innovation in this field have been transformed into applicable medical strategies and procedures with great benefits for patients. Approval of a test obligates its use. Missing guidelines and legitimate to identify centers and laboratories performing the SP have lead to more harm than good in some places.

There is an increasing need for a single national body which provides authoritative, objective decisions on whether, and under what circumstances new SP or diagnostic tests should be made available. Country's health system care, culture, demography and resources should be evaluated. Approval of a test should be accompanied by how the test might most efficiently be provided and when outdated tests can

be withdrawn.

Czech Republic have an old tradition in the field of prenatal screening programs with the first amniocentesis at 1971. Women aged 40 and than of 35 was the indications tell the late 80s. when the triple test have been introduced. Regular and detailed registration started at 1985. In the year 1990 the number of invasive testes were 3 % for about 20% detection rate for Down syndrome and 20 % other chromosomal abnormalities, in 1998 and 2006 it was 10% for 60 % and 18% for 80% respectively.

From this point of view a short description of the effect of implementing the new SP on our center and that over the country will be presented.

#### P03.18

##### Proteomic analysis of amniotic fluid in pregnancies with Turner and Klinefelter Syndrome fetuses

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**Objectives:** To determine the protein composition of amniotic fluid coming from pregnancies with normal, Turner and Klinefelter syndrome fetuses.

**Material & Methods:** Proteomic analysis was performed in stored amniotic fluid samples of eighteen 2<sup>nd</sup> trimester pregnancies. In five cases routine cytogenetic analysis had shown that the fetus had Turner syndrome, in four Klinefelter syndrome and in nine cases the fetal karyotype was normal (5 females and 4 males). Samples were analysed by Two-Dimensional Gel Electrophoresis (2DE), coupled with Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS). Selected proteins were further evaluated by Western blotting.

**Results:** As compared to controls TRFE, LUM, RETBP and ApoA1 were significantly increased in Turner syndrome cases, whereas KNG1, THBR and ApoA4 were decreased.

Four proteins (Apo A1, ZINCA2, LUM and A1AG) were found to be up-regulated in samples obtained from pregnancies with Klinefelter syndrome fetuses and three (RETBP, A1AT and VDBP) were down regulated.

**Conclusions:** Different sets of proteins were differentially expressed in the various sex chromosome abnormalities. Since these proteins are likely to cross the placenta and be detected in maternal plasma, if the specificity of our results is verified, they may be used as biomarkers for the noninvasive prenatal diagnosis of sex chromosome aneuploidies.

#### P03.19

##### Noninvasive prenatal diagnosis of fetal trisomy

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The discovery of fetal DNA as a small component of cell-free DNA in the maternal blood circulation has driven developments in non-invasive prenatal diagnosis (NIPD) for the past decade. Interest has focused upon NIPD of fetal trisomy 21, Down syndrome. We have explored DNA regions on human chromosome 21 with the aim of identifying DNA regions that are differentially methylated between adult leukocytes and placenta. These two tissues represent a model system for cell-free fetal and cell-free maternal DNA, respectively, in the blood plasma of pregnant women. Among 46 DNA regions analysed in this model system, a single differentially methylated region located in the *AIRE* gene promoter, was identified. Further analysis of the methylation of this DNA in the blood plasma of pregnant women indicated that its placental epigenetic signature was not consistently maintained in cell-free fetal DNA. The inconsistency exposes an apparent limitation of the adult leukocyte/placenta model system as a means of discovering epigenetic DNA biomarkers for use in the NIPD of fetal aneuploidies. Our data indicate that more direct strategies are required, using cell-free plasma DNA.

#### P03.20

##### Optimization of blood collection, fetal DNA isolation, concentration and analysis of fetal DNA present in maternal blood

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Invasive methods currently used in prenatal genetic diagnostics represent a not negligible risk for the health of both the subjected pregnant woman and fetus. Not surprisingly, the detection and analysis of fetal DNA in maternal plasma, representing a non-invasive alternative, attracted much attention in the past few years. In our study, we focused on comparing protocols for blood collection, isolation, concentration and detection of fetal DNA present in maternal plasma of pregnant women at various phases of pregnancy. We analyzed effects of addition or omission of 4% formaldehyde solution during blood collection and surveyed the implementation of DNA concentration after isolation procedure using either ethanol precipitation or vacuum concentration. We screened for presence of Y-chromosome specific sequences employing three different methods. Two were based on qPCR (one designed in our laboratory, one with commercial system specifically used for identification and quantification of degraded human DNA sequences) and one was based on PCR and fragment analysis of amelogenin loci on automated genetic analyzer. We have successfully prepared fetal DNA suitable for reliable qPCR and PCR analysis of Y-chromosomal sequences with all three tested detection systems. The comparison of the collection, isolation, purification and concentration approaches as well as detection methods can be observed in detail on our poster presentation.

#### P03.21

##### Early first trimester detection of fetal cells and DNA in maternal blood

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Detection of Y-chromosome sequences in fetal cell-free DNA or cells from maternal blood are useful tools in non-invasive prenatal diagnosis that have evolved into a fetal gender test feasible at earlier pregnancy stages compared to ultra-sound screening.

The goal of this study was to determine the earliest possible time-point for trophoblast and fetal cell-free DNA detection and determination of fetal sex as a model for comprehensive prenatal diagnosis. Maternal blood samples were collected from IVF patients at 2, 3 and 4 weeks post-implantation. Fetal sex was determined in cells with FISH probes following magnetic sorting with HLA-G, a trophoblast marker. Y-chromosome SRY sequences were detected in cell-free plasma DNA with real-time PCR. The results were compared with the newborn gender. Fetal sex was accurately determined in cell-free DNA in 12/16 (85%) of the samples, with 2 false-positive and 2 false-negative results. Trophoblast testing yielded 8/15 (53%) accurate fetal gender determination with 4 false-positive and 3 false-negative results. False-positive results probably reflect the presence of a non-developing male embryo as evidenced in one sample in which this explanation was supported by PGD analysis.

The IVF samples yielded less accurate results compared with our ongoing study of late 1<sup>st</sup> trimester samples (weeks 7-13, parallel to 5-11 weeks post-implantation) in which 98% accuracy of fetal sex determination was achieved with cell-free fetal DNA and trophoblast methods. The uniqueness of the sample group - IVF patients - in which multiple embryos are usually implanted, and the early stage of pregnancy, can explain this discrepancy.

**P03.22****Contamination-free analysis of single cells in cell-based non-invasive prenatal diagnosis**

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Analysis of very rare cells, as this is true for non-invasive prenatal diagnosis (NIPD) based upon fetal cells circulating in the peripheral blood of pregnant women present a formidable challenge in respect of the need for non-ambiguous data. Currently used marker lack the potential to exclusively discriminate fetal from maternal cells due to unspecific staining, lack of fetal specificity, or low signal intensity. Thus, pooling of fetal cells to enhance analysis efficiency in subsequent molecular genetic analysis may give ambiguous data due to contamination with maternal cells.

To circumvent maternal contamination while using the advantage of pooling cells to increase analysis significance we are working on a procedure to define a Post Identification Pool (PIP). The rationale is to use markers with inherently limited specificity to (semi-automatically) detect fetal candidate cells that are laser-microdissected and forwarded to single cell whole genome amplification (scWGA). Aliquots of the latter are then analysed by means of DNA fingerprinting using fluorescent multiplex PCR. ScWGA products whose DNA profiles differ from the maternal control sample are then pooled for further analysis.

Single fetal and maternal cells derived from one and the same interruption material were subjected to PowerPlex 16 System® proofing the DNA fingerprinting to be sufficient for discriminating fetal from maternal cells. Currently a modified 16-fold multiplex is being set up to perform DNA fingerprinting on pre-amplified (scWGA) single cells to implement the PIP procedure.

**P03.23****Clinical practice of the incorporation of the non-invasive fetal gender assessment in maternal blood**

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Prenatal diagnosis is currently recommended to those pregnancies at risk of an X-linked disorder however the invasive obstetric procedures required entail a risk of miscarriage. Early fetal sex determination in maternal blood can avoid the need for conventional PD in a half of these cases. A previous large-scale validation study performed in our laboratory concluded that this methodology was 100% accurate from the 7<sup>th</sup> week of gestation.

After incorporating this analysis into the clinical routine we have diagnosed a total of 32 pregnancies at risk of an X-linked disorder including cases of Haemophilia, Duchenne Muscular Dystrophy, Norrie Disease... Two plasma samples were collected from each pregnant woman in the first trimester of gestation: one from the 7<sup>th</sup> week of gestation and another from the 9<sup>th</sup> week. Fetal gender was determined by the presence/absence criteria of the SRY gene by Real-Time PCR. Conventional prenatal diagnosis, ultrasound scan or gender at birth confirmed that fetal sex was correctly diagnosed in maternal blood in all cases. Early diagnosis of fetal sex in maternal blood represents a great advantage for pregnancies at risk of an X-linked disease because invasive prenatal diagnosis is suppressed in a half of the cases. Since this diagnosis is performed before the 12<sup>th</sup> week of gestation, chorion villus sampling can be done in the case is required.

**P03.24****Non invasive prenatal detection of two RHD gene exons and fetal sex using cell free fetal DNA in maternal plasma**

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Cell free fetal DNA (cffDNA) detection by Real-time PCR is routinely applied for non-invasive genotyping of the fetal RhD status in reference laboratories. The assay is easy to automate allowing high throughput.

We developed a new rtPCR for non-invasive prenatal RHD genotyping and fetal sex determination using maternal plasma.

Two Taqman MGB-probes and primers were designed to develop a Multiplex rtPCR for simultaneous amplification of exons 5 and 7 on RHD gene. The multicopy DYS14 sequence on the Y chromosome was also included in the assay. The test was evaluated blind on 50 coded plasma samples of known fetal genotype obtained from RhD negative pregnant women, archived in our lab at -20°C over the last two years.

DYS14 products were detected in all 28 samples from male fetuses; both RHD exons 5 and 7 were detected in 39 samples (23 males and 16 females). No false positive were observed. Absence of all three products indicating female RhD negative fetuses was observed in 6 cases. Fetal sexing results were 100% concordant, only in one sample RhD exons failed to amplify resulting in an RhD negative female fetus.

Even in old archived plasma samples multiplex rtPCR detection of cffDNA was efficient and reliable allowing the assessment of fetal sex in all cases. Only one sample from a RhD<sup>+</sup> female fetus was genotyped as RhD<sup>-</sup> probably because of cffDNA degradation derived from repeated thaw freezing cycles of the original plasma. The procedure proved to be sensitive enough to be applied on clinical cases.

**P03.25****Simple and fast isolation of cell-free circulating DNA from human plasma and serum**

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The discovery of cell-free circulating DNA in plasma opened up interesting possibilities for noninvasive prenatal diagnosis as an alternative to established invasive genetic screening procedures such as amniocentesis and chorionic villus sampling. However, the isolation of circulating DNA from plasma or serum is challenging. Circulating DNA is highly fragmented and of very low concentration. Thus, established nucleic acid purification protocols and ready-to-use kits have only a very limited suitability for the extraction and purification of circulating DNA. To overcome these limitations we developed the *NucleoSpin Plasma XS* kit specially designed for the isolation of fragmented DNA ≥ 50 bp from human EDTA blood plasma. The kit exploits the benefits of a unique binding column with a minimised dead volume and allows for elution in 5-20 µl. Using up to 240 µl plasma the kit offers an easy and convenient way for efficient purification of circulating DNA from plasma. Data from kit development as well as application data, e.g., from fetal Rhesus D typing will be presented.

Besides prenatal genetic testing cell-free circulating DNA promises to be applicable for the screening and assessment of a variety of pathological findings such as cancer, stroke, myocardial infarction, inflammation or trauma. The use of the *NucleoSpin Plasma XS* kit for these applications will be exemplified.

**P03.26****Update of ESHRE PGD Consortium Activities**

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Since 1997, the European Society of Human Reproduction and Embryology (ESHRE) Preimplantation Genetics Diagnosis (PGD) Consortium has collected technical and outcome data, provided referral networks, surveyed and promoted best practice. Membership increases steadily (n=91) with increasing numbers of centres reporting (16-45) and cycles reported (392-3358) between reports 1 and 7. During this time, reported cycle numbers for constitutional chromosome abnormalities and monogenic disorders have increased with disproportionately larger increases in preimplantation genetic screening (PGS) cycles reflecting the increasing tendency for IVF laboratories to select the 'best' embryo for transfer by elimination of chromosomally abnormal embryos. Methodologies for every technical aspects of PGD are becoming more sophisticated, accurate and reliable ensuring extremely low misdiagnosis rates. PGD babies are comparable to those derived from IVF with intracytoplasmic sperm injection procedures with respect to pregnancy complications and congenital malformation. The main complication, as with routine IVF, remains the risk of multiple pregnancy and concomitant higher morbidity and mortality.

The Consortium has published best practice guidelines for PGD and PGS and recently, a joint report with the European Society for Human Genetics (ESHG) broadly examining the interface between genetics and assisted reproductive technology. To further improve preimplantation testing, studies are ongoing to investigate PGD laboratory accreditation, appropriate external quality assessment, EQAS (multicentric evaluation of captured FISH images from single embryonic nuclei) and measures to facilitate real-time reporting of misdiagnoses and development of preventative action to reduce the risk of recurrence. Survey data regarding misdiagnosis, EQAS and confirmation of diagnosis on embryos will be shared.

#### P03.27

#### Pre-implantation Genetic Diagnosis (PGD) for Genetic and Metabolic Disorders-The Saudi Experience

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Saudi Arabian culture is highly consanguineous, with the first cousin marriages accounting for 60-70% of all marriages. Given the difficulties in management of genetic disorders, reproductive option for families affected with genetic diseases in Saudi Arabia is often limited to PGD which is permissible under the law and religion whereas prenatal diagnosis with the intent of termination of pregnancy is neither widely practiced nor socially accepted, although it is accepted under certain conditions.

KFSH&RC has been offering PGD for monogenic and chromosomal disorders, since 2001. A total of 45 pregnancies initiated. Of which, 25 healthy babies were born, 13 pregnancies are ongoing and 7 pregnancies were either biochemical or ended up with abortion.

In all these families PGD was undertaken using fluorescent PCR (F-PCR) and/or nested PCR with sequencing on a single cell, or Multiple Displacement Modification (MDA) to amplify the whole genome from a single cell. A singleton pregnancy ensure after transfer of one heterozygous and one/or normal embryo and prenatal diagnosis by CVS confirmed a normal pregnancy. This is the first report of successful PGD in different genetic disorders in Saudi Arabia, and the Muslim world.

#### P03.28

#### Preimplantation Genetic Haplotyping for Duchenne/Becker muscular dystrophy-birth of first male babies

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Preimplantation Genetic Diagnosis for sex-linked disorders undertaken by sexing embryos is contentious as approximately 50% of the male embryos discarded will be free of the mutation. Direct diagnosis of dystrophin mutations is technically challenging due to heterogeneous mutations and recombination hotspots. Since January 2006, we have offered an alternative approach for Duchenne/Becker muscular dystrophy (D/BMD) using Preimplantation Genetic Haplotyping (PGH). The first 10 cases (9 DMD + 1 BMD) are presented with follow-up to February 2008. In this cohort, 7 of the carrier females had different exonic deletions, whilst 3 had different point/micro mutations, including a germline mosaic. 15 microsatellite markers spanning the gene were used, alongside 5 Y-chromosome markers to detect possible sex chromosome aneuploidy.

57 embryos were biopsied with 50 diagnosed (88%) and 32 (56%) suitable for transfer. The multiple displacement amplification (MDA) used in PGH had a significantly lower ( $p<0.0001$ ) allele drop out in male (23.1%) than in female (38.8%) embryos (average 29.9%). 11% recombination was observed but the density of markers allowed accurate location of the cross-over relative to mutation positions. All 10 cycles achieved embryo transfer with a 70% pregnancy rate and 50% clinical pregnancy rate resulting in 5 deliveries of 3 normal females, 1 carrier female and 2 normal males.

This successful PGH approach to X-linked disease is also being applied to Alport's syndrome, Haemophilia A and B, Fragile X, Adrenoleucodystrophy and Hydrocephalus.

#### P03.29

#### A new mutation in the AFP gene responsible for a total absence of alpha feto-protein on 2nd-trimester maternal serum screening for Down syndrome

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#### Introduction

Alpha feto-protein (AFP) is a major plasma protein produced by the yolk sac and the liver during the foetal period. During the 2nd-trimester of pregnancy, AFP combined with  $\beta$ hCG are commonly used for Down syndrome risk evaluation. Deficiency of AFP is a rare phenomenon (estimated to occur in 1/105000 newborns) and only one sequence alteration in *AFP* gene was reported. Here we report a new mutation in exon 5 of the foetal *AFP* gene leading to a total absence of AFP on 2nd-trimester maternal serum screening for Down syndrome, confirmed on the amniotic fluid. Despite this, foetal development and birth were normal.

#### Methods

After PCR-amplification, the whole *AFP* gene was sequenced. To determine the amniotic fluid profile, proteins were separated by electrophoresis and compared with 10 normal amniotic fluids sampled at the same gestational age (18 weeks).

#### Results

The new mutation observed was a guanine to adenine transition in position 543 creating a premature stop codon in position 181. A new SNP was characterised in the exon 12 (c.1641A>G). Albumin rate in the amniotic fluid was reduced compared to controls, whereas alpha1 and beta protein fractions were increased.

#### Discussion - Conclusion

The c.543G>A mutation is the second sequence modification identified so far in the *AFP* gene, responsible for its complete deficiency. Electrophoresis results suggest that deficiency may modify protein fraction repartition, particularly albumin, alpha1 and beta fractions suggesting complex molecular mechanisms of compensation. Studies on other families with AFP deficiency are necessary to confirm this observation.

#### P03.30

#### The evaluation of chromosomal abnormalities diagnosed prenatally in Cluj-Napoca,Romania

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Prenatal diagnosis for chromosomal disorders is performed routinely in populations since most of these disorders have severe consequences such as major malformations and mental retardation. Since advanced technologies in rapid diagnostic tests have been developed to detect common trisomies prenatally it is essential that each laboratory should evaluate their own prenatal diagnosis profile. In this study we aimed to investigate the type and proportion of chromosomal abnormalities detected in cytogenetic studies prenatally and referral indications in 684 pregnant cases in Cluj-Napoca,Romania between the period of 2002-2007. The overall chromosomal abnormality rate was found to be 49/684(7.76%). The cytogenetic analysis with GTG banding of amniotic fluid cells revealed: trisomy 21(12 cases), trisomy 18(7 cases), monosomy X(6 cases), trisomy 16(1 case), trisomy 8(1 case), trisomy 15(1 case), Robertsonian translocations(2 cases), Klinefelter syndrome(3 cases), autosomal deletions(3 cases), autosomal monosomy(2 cases), poliploidy(3 cases), chromosome marker(6 cases), trisomy X(1 case), Fra 5q31(1 case). Careful genetic counseling by expert geneticists regarding the patients indications is essential for determining the cost-effective prenatal diagnostic test for each patient.

#### P03.31

#### Prenatal expression of Baraitser-Winter syndrome

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Baraitser-Winter syndrome was first reported by these two authors in 1988 in a brother and a sister, and a girl unrelated to the two others. The three patients had iris coloboma, hypertelorism, ptosis, mental re-

tardation, large nasal root, and epicanthus. Two similar observations with pericentric inversion of chromosome 2 were reported. In 1995, the possibility of gyration abnormalities and trigonocephaly was noticed. Due to the initial familial observation, an autosomal recessive mode of inheritance was suggested.

We report the observation of a young boy, presenting with this condition. His prenatal history was marked by hygroma colli with normal karyotype, persisting along the pregnancy, associated from the second trimester with renal dilatation and intestinal hyperechogenicity. At birth, Noonan syndrome was first considered. *PTPN11* analysis was performed but negative. At the age of 4, due to suggestive dysmorphic features, the diagnostic of Baraitser-Winter syndrome was made. However the patient had no iris coloboma or gyration abnormality. We discuss the similarities with Noonan syndrome, even during the prenatal period.

#### P03.32

##### Prenatal detection and molecular characterisation of two supernumerary marker chromosomes (SMC) derivatives of chromosome 22 in two unrelated patients.

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SMC are 'additional markers' whose origin and composition cannot be determined by conventional cytogenetics. Genetic counseling of patients with SMC can be difficult, especially in prenatal testing, due to the complexity in establishing a karyotype-phenotype correlation. In fact, it has been estimated that about 37% of prenatal diagnosed SMC are associated with an abnormal phenotype. We report two cases of prenatal diagnosed SMC, detected by G-banding and completed by C-banding and NOR-staining. In the first case, the marker was familial, segregating from a mother with non-mosaic karyotype. In the second case, the SMC was de novo and present in the 78% of cells. The following FISH procedures were performed to confirm and specify the material present in the marker chromosomes: locus-specific identifier, 24-colour FISH, CGH, chromosomal manual microdissection and reverse FISH. These techniques identified both SMC as derivatives of chromosome 22. In the first case, the inherited SMC was an iso(22p) and the child was delivered successfully without phenotypic abnormalities. In the second case, the SMC was a der(22)(q11.2), resulting in a partial trisomy of band 22q11.2, which has been associated with Cat-eye syndrome (CES) in the literature. In this case the pregnancy was interrupted. It can be concluded with this study that familial markers representing an iso(22p) can be correlated with a normal phenotype. Also, this work proves the importance of the application of molecular cytogenetic procedures to know the origin of de novo SMC, in order to give accurate prenatal genetic counseling.

#### P03.33

##### Cell-free DNA levels during labor at term and preterm deliveries

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The aim was to evaluate the effect of uterine contractions on cell free fetal-DNA (cff) and total DNA levels in maternal plasma during term and preterm deliveries. Further we determined whether the cff-DNA concentration from maternal plasma samples during deliveries at term are different from those with preterm deliveries.

Maternal venous blood samples were collected during deliveries from 65 pregnant women with male fetuses. 46 pregnant women delivered at term and 19 delivered preterm. Cff-DNA levels were analyzed by quantitative real-time PCR using the SRY-assay and total DNA levels were analyzed using the beta-Globin-assay. 148 pregnant women at term without labor served as controls for women in labor at term. Preterm samples (>27wks, 28-31wks, 32-36 wks, >36 wks) were matched for gestational age to control samples without labor.

Cff-DNA levels in plasma samples from women during delivery at term were statistically higher than fetal DNA levels in women without labor at term ( $p=0.014$ ). The same results were found in the groups of preterm deliveries (28-31wks:  $p=0.048$  and 32-35wks:  $p=0.003$ ). A statistically significant difference was not found in the lower age group (>27wks) which was probably due to the low number of samples. Furthermore, a statistically significant increase of fetal DNA during deliveries at term was found when compared to preterm deliveries ( $p=0.05$ ).

Cff-DNA is increased during spontaneous deliveries at term and preterm when compared to pregnant women without labor. Therefore, cff-DNA levels might be used as a marker for preterm deliveries.

#### P03.34

##### Prenatal diagnosis of chromosomal abnormalities in Tomsk population

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The aim of current study was to evaluate the frequency and structure of prenatally detected chromosomal abnormalities in Tomsk population. The risk for chromosomal aberrations was calculated by taking into account maternal age and gestation time, first and/or second trimester serum markers, first and/or second trimester ultrasound. Cut-off for invasive testing was risk 1 in 250. A total of 1349 chondrocenteses, amniocenteses, placentocentesis and chorion biopsies were performed during the period of 5 years. Karyotype clarification was performed by conventional cytogenetic analysis. Abnormal karyotypes were found in 4.8% of cases. Numerical and structural chromosomal aberrations were detected in 73.8% and 26.2% of cases, respectively.

#### P03.35

##### Chromosome abnormalities in amniocenteses with normal fetal ultrasonography

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Before 1994, maternal serum screening (MSS) was not offered to women 35 years of age or older, unless they did not choose to undergo invasive diagnostic testing for chromosomal abnormalities. MSS tests are usually performed for detecting trisomy 21 in women under 35 years of age. Their use in women 35 years of age or older has a detection rate of 67% for all chromosomal abnormalities and 87% for trisomy 21. In our study, we evaluated karyotype results of amniocenteses of women with advanced maternal age (AMA), increased risk for trisomy 21 on MSS (MSS-DS, first and/or second trimester tests) and with AMA+MSS-DS. None of the patient groups had fetal abnormalities detected during ultrasound examination. Among 2231 patients consulted between January 2002 and December 2007, 52 (2.33%) had chromosome abnormalities. 20 of 817 cases (2.44%) with AMA, 23 of 1121 cases with MSS-DS risk (2%) and 9 of 293 cases with both AMA and MSS-DS risk among (3.07%) had chromosome abnormalities. We detected trisomy 21 in 23 cases (7 AMA, 8 MSS-DS and 8 AMA+MSS-DS), mosaic trisomy 21 in one case with MSS-DS, mosaic trisomy 20 in one case with AMA, sex chromosome aneuploidies in 9 cases (6 AMA, 3 MSS-DS), unbalanced rearrangements in 3 cases (1 AMA, 2 MSS-DS), marker chromosome in 3 cases (1 AMA, 2 MSS-DS), balanced rearrangement in 12 cases (4 AMA, 7 MSS-DS, 1 AMA+MSS-DS). Although the fetuses had normal findings in fetal ultrasound examination, pregnancies with increased risks still had chromosome abnormalities.

#### P03.36

##### Hypertelorism-Microtia-Clefting syndrome, two further prenatal cases

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<sup>1</sup>Jeanne de Flandre, Lille, France, <sup>2</sup>Centre de Biologie Pathologie, Lille, France. Hypertelorism-Microtia-Clefting syndrome (HMC syndrome) is a very rare autosomal recessive condition, with only nine cases reported in the literature. It comprises hypertelorism, microtia, cleft lip and/or palate, microcephaly, variable mental retardation, renal anomalies and sometimes heart abnormalities.

Here we describe two affected foetuses.

During the first pregnancy, an increased nuchal translucency was identified at 12 WG. Chromosomes on Chorionic Villus Sample (CVS) were normal 46, XX. Secondly, heart abnormalities and a bilateral cleft lip and palate were noted.

This multiple congenital anomalies syndrome led to termination of pregnancy at 20 WG. Pathology examination revealed severe hypertelorism, microtia, bilateral cleft lip and palate.

HMC syndrome was suspected and since it is an autosomal recessive inherited defect, the genetic counselling was cautious. We recommended close detailed ultrasound follow-up for further pregnancies.

During the second pregnancy, a recurrence was observed. Indeed, the foetal ultrasound showed at 12 WG an increased nuchal translucency and a right cleft lip. Chromosomes on CVS were normal 46, XY. Pathology processed after the termination of pregnancy at 16 WG revealed hypertelorism, small ears and a right cleft of the upper lip.

The parents are non consanguineous. Their karyotypes are normal. A CGH-array study is pending on the first foetus. So far no responsible gene is known for the HMC syndrome and the hypothesis of chromosomal abnormality remains plausible.

#### P03.37

#### First successful prenatal diagnosis of MDC1A form in Tunisia revealed intrafamilial phenotypic variability in two siblings sharing the same mutation in LAMA2 gene

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MDC1A is a severe congenital muscular dystrophy caused by mutations in LAMA2 gene encoding the laminin  $\alpha 2$  chain. Prenatal diagnosis represents prevention for many couples given the overwhelming prospect of having another child with this incurable condition. We report the first prenatal diagnosis of MDC1A form in Tunisia and in Africa in a family with previously affected child and identified mutation in LAMA2 gene. Amniotic fluid was sampled by amniocentesis under ultrasound guidance. Molecular analyses were performed on cultured amniotic fluid cells after exclusion of maternal cell contamination by QFPCR. Postnatal clinical examination was also performed by cerebral MRI and by immunostaining on muscle biopsies using two monoclonal antibodies directed against the laminin  $\alpha 2$ . After exclusion of maternal cell contamination, mutation screening on fetal DNA showed that he was homozygous for the c.8007delT frameshift mutation, and the couple was counselled that the foetus would be affected. The presence of the mutation was confirmed on total DNA extracted from blood leukocytes of the newborn. Surprisingly, postnatal clinical examination showed that the younger patient who was diagnosed as affected developed widely milder phenotype of MDC1A form than his severely affected brother; and immunfluorescence showed complete deficiency of the laminin  $\alpha 2$  in both patients. These findings suggest that other genetic/or epigenetic factors including modifier gene can control the course of the disease. Moreover, the intrafamilial phenotypic variability in siblings with the same molecular defect complicates the diagnoses because presymptomatic LAMA2 mutation carriers can develop a different phenotype than previous diagnosed porosities.

#### P03.38

#### Indications for cordocentesis and correlation with chromosomal aberrations

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Over a 7 years period we performed 734 cytogenetic analysis of foetal blood taken by cordocentesis. Cordocenteses was performed because of late gestation. Indications for cordocentesis were advanced maternal age, increased risk determined by biochemical screening or sonographically detected foetal abnormalities. Indications and findings are given in table 1.

Table 1.

	Total	Aberrant	%
Biochemical Screening Risk	181	2	1,1%
Advanced Maternal Age	249	18	7,2%
Polyhydramnion	79	10	12,7%
Oligohydramnion	50	2	4%
IUGR	111	4	3,6%
CNS Anomaly	48	3	6,3%
Foetal Hart Defect	16	4	25%
Total	734	43	5,85%

In nearly 6% of analysed samples we found aberrant karyotypes. The most common finding was autosomal aneuploidy. Trisomy 21 correlated with advanced maternal age, polyhydramnion and foetal hart defects, while trisomy 18 was predominately found in foetuses with CNS abnormalities. Structural chromosomal abnormalities were detected only in 5 cases (0,7%), and had different phenotypic expression.

#### P03.39

#### Case Report: Submicroscopic duplication of D18S535 in a fetus with normal karyotype

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QF PCR (Quantitative Fluorescent PCR) was introduced only a few years ago to speed up prenatal diagnosis and act as an adjuvant to standard karyotype analysis. QF PCR utilizes length polymorphisms of Short Tandem Repeats (STR) on selected chromosomes that are amplified and then detected by automatic genetic analysers. STRs are found in abundance in the human genome and their usefulness as potential forensic markers for human identification purposes with the help of PCR has been noted almost 20 years ago. Many STRs have been utilized in the field of prenatal diagnosis leading to the development of QF PCR (Quantitative Fluorescent PCR) with the concurrent advance of technology in genetic analyzers. Here we report a case of a submicroscopic duplication of STR D18S535 in an amniotic fluid sample screened routinely for aneuploidies. This partial trisomy involving the chromosome 18 was diagnosed by detecting this pattern only in one out of three markers used for chromosome 18. The rest of the STRs of chromosome 18 tested were normal. After screening the parents it was found that the extra allele had been inherited by the father so the pregnancy continued normally. Conventional cytogenetic analysis that was performed on this amniotic fluid had a normal karyotype. There have been reports of other such duplications in the literature and stringent screening is required to avoid false positive results when screening for aneuploidies.

#### P03.40

#### Prenatally detected trisomy of chromosome 21

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We present incidence of prenatally detected trisomy of chromosome 21 during last seven years (2000-2007) in Medical Genetic Centre in Novi Sad, Vojvodina northern part of Serbia with 2.000.000 inhabitants.

Suspicion for trisomy 21 and indications for invasive prenatal diagnosis was made by Clinical Genetics according maternal age (35 and more), paternal age (42 and more), family pedigree, biochemical screening, expert ultrasound result such as enlarged nuchal translucency, absent nasal bone, echogenic bowels, short femur, and other.

During last seven years we detected 99 trisomy of chromosome 21 (full trisomy 91 cases, mosaicism 5 cases, translocations 3 cases), that is 38.98% of all prenatal detected chromosomal anomalies (N=99/254; 38.98%).

#### P03.41

#### Screening for different Chromosome 18 methylation patterns between placenta and whole blood

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Since its discovery in 1997, the presence of free fetal DNA in maternal plasma provided new approaches for non invasive prenatal diagnoses.

However, universal fetal markers would allow its diffusion to a wider number of applications and clinical cases.

More recently epigenetics has shown encouraging results toward this goal. Our aim was to investigate the methylation status of selected promoter regions of chromosome 18 genes in maternal blood and placenta samples.

A total of 36 genes on chromosome 18 were selected after database search for sequences with different expression patterns in placenta and blood. Primers were designed to analyse as much CpG islands as possible in 102 promoter sequences for the presence of different methylation patterns in fetal tissue (CVS) and whole blood (WB). Digestion with 4 methylation-dependent restriction enzymes was carried out on extracted DNA samples prior to PCR amplification to investigate the presence of differentially methylated sequences.

Two promoter regions were found hypermethylated in CVS and hypomethylated in WB. One more gene promoter was found with the opposite pattern being hypomethylated in CVS and hypermethylated in WB.

In this study we found three genes on chromosome 18 with apparently differentially methylated promoters between placenta and whole blood. We are in the process of developing assays to detect the placental form in maternal plasma. These preliminary results confirm the effectiveness of an epigenetic approach to find biomarkers for the analysis of free fetal DNA in maternal plasma.

#### P03.42

##### **Screening and nuchal translucency in first and second trimester: efficiency in the selection of pregnant women at risk of chromosomopathies**

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First trimester screening for trisomy 21 is provided by a combination of maternal age, fetal nuchal translucency and maternal serum free β-hCG and PAPP-A concentration between 11-13 weeks of gestation. The detection rate of trisomy 21 is 90% with a false positive rate of 5% which is superior to the 65% achieved by second-trimester serum biochemistry. We report a series of 5285 gestations that were referred to maternal-fetal medicine department between 2003 and 2007 who underwent an invasive prenatal diagnosis. A total of 1986 samples were CVS and 3299 were amniotic fluid samples. In first trimester the 12,7% of the samples were referred due to positive combined screening (+SC) and the 13,7% due to increased nuchal translucency (INT) as unique abnormal parameter. In second trimester the 39% of the samples were referred for positive second trimester screening and the 2,5% for increased nuchal translucency. In the second trimester samples the 1,4% of +SC and the 9,3% of INT showed a chromosomopathy. In the first trimester samples the 12% of +SC and 25% of INT presented chromosomal abnormalities. In first trimester we must perform 5,5 invasive procedures to find a chromosomopathy in group of patients with the reported indications, whereas in the second trimester 53 invasive procedures are needed to detect a chromosomal abnormality. These results emphasise the higher efficiency of first trimester screening over the second besides the medical advantages of an early diagnosis.

#### P03.43

##### **First-trimester screening protocols and their impact in cytogenetic counselling**

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In the last few years, we have referred an increase of diagnosis prenatal (DP) due to advanced maternal age and more knowledge of the general population about possible diagnosis methods. The assumption of first-trimester screening protocols (ultrasound and biochemistry methods) has allowed to generalize the DP to all pregnant women, independently of maternal age. We present our results after seven years with the combined test and its impact on the cytogenetics prenatal diagnosis. We analyzed data from 5743 singleton pregnancies under-

going prenatal genetic counselling from the area 7 of Madrid. 4930 woman where testing by first-trimester combined biochemistry and ultrasound nuchal translucency screening. In 1998, 13% of the pregnant women renounced amniocentesis opposite a 50% of renounces in 2007. The implementing of first-trimester screening has reduced the number of amniocentesis and made an individual better earlier genetic counselling.

#### P03.44

##### **Prenatal Diagnosis of a fetus with 44 chromosomes and homozygous Robertsonian translocations (14;21)**

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We report the rare finding of a human fetus with 44 chromosomes due to homozygous Robertsonian translocations (14;21). The related parents, born in Turkey, were referred to our Genetic Department for genetic counselling at 16th weeks of gestation due to advanced maternal age. Amniocentesis was performed and the analysis of amniotic fluid cells revealed 44 chromosomes with a homozygous Robertsonian translocations (14;21):

44,XX,der(14;21)(q10;q10),der(14;21)(q10;q10). At the time of amniocentesis there were no pathological ultrasound findings. Karyotyping of the consanguineous parents showed in each case the same heterozygous Robertsonian translocations(14;21). Family history revealed that the translocation has segregated through at least four generations. Because UPD testing should be considered in fetuses carrying a balanced Robertsonian translocation involving chromosomes 14 or 15, we excluded in our case UPD 14. Although Robertsonian translocations are common chromosomal rearrangements, homozygous carriers are rare. Previously reported single case reports showed no evidence for an increased risk for malformations or dysmorphic features in carriers of homozygous Robertsonian translocations.

#### P03.45

##### **Prenatally detected mosaicism - 46,X,idic(Y)(q11.2)/45,X. A case report**

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Isodicentric Y chromosomes are inherently unstable and may be lost during mitosis resulting in mosaicism, generally including a 45,X cell line. Clinical presentations in patients with mosaicism for a structurally abnormal Y chromosome can range from classical Turner phenotype through mixed gonadal dysgenesis to phenotypically normal males.

The variability in sexual phenotype is thought to be related to the tissue distribution, to the proportion of each cell line and to the location of the breakpoints. However, prenatal diagnosis of mosaic 46,X,idic(Y)/45,X is rare, and poses a serious dilemma concerning the prognosis related to the fetal stature, sexual differentiation and the risk development of gonadoblastoma.

In our case amniocentesis was performed to a 33-year-old women because of positive serum screening on the 15 week of pregnancy. Cultured amniocytes from two different culture flasks revealed mosaic karyotype 46,X,+mar[36]/45,X[14]. For detecting the origin of marker chromosome, fluorescence in situ hybridisation (FISH) was performed 1)with X and Y centromeric probes (AneuVysis, X, Y-alpha satellite probe) and 2) XYpter subtelomere specific probe (Cytocell). FISH analysis revealed that the marker chromosome was isodicentric Y chromosome with two short arms and a small portion of the long arm: 46,X,idic(Y)(q11.2)[41]/45,X[44]. After further genetic counselling, the parents opted for termination of the pregnancy. Postmortem examination revealed male fetus with microanomalies and no major abnormalities. Cytogenetic analysis of the placenta showed mosaicism in a different degree 46,X,idic(Y)(q11.2)[46]/45,X[4] and mosaicism was also found in fetal tissues.

#### P03.46

##### **Is informed choice in the context of prenatal testing a universally held value?**

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Background: Informed choice is central to prenatal testing and endorsed by many national and international guidelines for prenatal

service delivery. Recent evidence suggests that there is considerable variation in the extent to which these guidelines translate into practice. This is particularly evident in cross-country comparisons. This paper presents findings from a values survey that assessed the extent to which informed choice in the context of prenatal testing is a universally held value.

**Method:** Values attached to choice in the context of prenatal diagnosis were assessed among 1) general populations ( $n=1200$ ) and 2) obstetricians ( $n=1117$ ) in Europe (UK, Netherlands, Italy and Greece) and Asia (China and India).

**Results:** Findings from both samples suggest that there is a sharp divide between first Northern European countries and second, Southern European and Asian countries in the extent to which choice is valued. Most respondents from Northern European countries perceived that undergoing prenatal testing should reflect a parental choice (71%-86%), compared with a minority from Southern European and Asian countries (16%-33%). Comparison across the populations suggests that health professionals are more strongly in favour of choice than general populations. Given the political endorsement of informed choice, the latter may reflect a tendency for professional identities to dominate over personal values.

**Conclusion:** Implications of these findings for policy and practice will be discussed within the framework of the ethical principle of 'autonomy'.

#### P03.47

#### Couple's karyotyping in recurrent miscarriage: A case report with t(13;14)

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An individual who is a carrier of a translocation may not have any problem with their growth, development and health but depending on the chromosomes involved, they may experience reproductive problems such as infertility, miscarriage and having a child with abnormal chromosomal complement. In the present study, chromosome study was carried out on young couple who was married for 1.5 yrs and had three consecutive missed abortions. However, chromosome study was not carried out in any of the abortus. Couples karyotyping following PHA-stimulated blood culture detected constitutive abnormality in husband with 46,XY,t(13;14)(q22.1;q24.3) pattern. The translocation between the two chromosomes was a balanced one with no significant phenotypic effect. The wife had a normal female karyotype. The possible genetic make up of the male gametes and subsequently of the fetus could be: i) both normal 13 and 14, ii) rearranged 13 with normal 14, iii) rearranged 14 with normal 13, and iv) both rearranged 13 and 14. Mathematically, she has a 1 in 4 (25%) chance of having baby entirely chromosomally normal and the others will be a translocation 'carrier' just like father or carrier of a recombinant 13 or 14. Therefore, 3/4 of her babies are at risk for being chromosomally abnormal. These babies could have severe congenital malformations and if they manage to survive birth and the neonatal period, profound metabolic disturbances and mental retardation could persist life long. However, prenatal diagnosis in CVS or amniotic fluid must be carried out for evaluation of chromosomal composition in fetus and obstetric management accordingly.

#### P03.48

#### Triploidy identified through Maternal Serum Screening in Second Trimester

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Amniocentesis was carried out at 17 weeks gestation on a 27 years old woman following an abnormal maternal serum screening test (MSS). MSS test was carried out primarily to estimate the risk of trisomy for chromosomes 21 and 18. The maternal serum markers used were alpha-fetoprotein (AFP), human chorionic gonadotrophin (hCG), and unconjugated estriol (UE3) together with maternal age. The fetus was identified as screen-positive for Edward's syndrome (trisomy18), with low UE3, normal AFP and hCG levels. The calculated risk for trisomy 18 was more than 1:50.

To identify any possible chromosomal abnormality, cytogenetics investi-

gation was carried out on amniotic fluid sample. The fetus's karyotype was triploid with 69, XXX chromosome complement in all the metaphase spreads obtained from three different cultures, using GTG banding technique. Upon termination of the fetus, gross abnormalities indicative of triploidy were present in the fetus.

This is one of a few cases reported so far where abnormal maternal serum screening indicative of trisomy 18, ends up as a triploid fetus. Maternal serum screening is an essential approach for detecting chromosome abnormality prenatally, and thus helping with prevention of the birth of a genetically abnormal fetus.

#### P03.49

#### A new prenatal diagnosis of Nager syndrome on monochorionic-biamniotic twin pregnancy

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The Nager syndrome, also termed preaxial acrofacial dysostosis, is a rare condition characterised by limb and facial deformities. Limb anomalies consist of hypoplasia or absence of radius, radioulnar synostosis, and hypoplasia or absent thumbs. The mandibulofacial dysostosis is characterised mainly by severe micrognathia and malar hypoplasia.

Since its first description in 1948, more than 80 cases have been reported in the literature, although there are only two reports of prenatal diagnosis. We report here a further case of Nager syndrome diagnosed at 22 weeks of gestation in a monochorionic biamniotic twin pregnancy. The prenatal diagnosis was performed on ultrasound because of severe microretrognathia and significant forearm shortening on both foeti. Termination of pregnancy was performed at 25 WG. Chromosomes were normal 46XY. The pathology report confirmed the diagnosis of Nager syndrome in both twins. Growth discordance was noted between the fetuses. Both presented a large cleft palate, ear dysplasia and a severe microretrognathia. They had bilateral thumb agenesis with single palmar crease. The X-ray confirmed the microretrognathia. One foetus had bilateral radioulnar synostosis with hypoplasia of the right radius, and bilateral thumb agenesis. The other twin presented bilateral thumb agenesis too, but only a partial radioulnar synostosis on the left side. A CGH-array analysis on fetal DNA is pending.

#### P03.50

#### Cytogenetic analysis of chorionic villi samples (CVS) in missed abortions

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The aim of our study was to analyse the frequency and type of chromosomal aberrations of chorionic villi samplings (CVS) in patients with missed abortion, performed in our clinic during last three years.

**Methods:** CVS analyses obtained by transabdominal biopsy in first and second trimester of 57 pregnant women with ultrasonographic evidence of missed abortion were performed. For chromosome analysis conventional cultures of CVS and GTG-banding techniques were used.

**Results:** Of total 579 CVS analysed, 57 CVS were performed for missed abortion. The mean maternal age was 35,86 (22-46) years, and mean gestational week was 9,75 (8-16). Fetal karyotyping was successful in 54 cases, while 3 cases were excluded from study for low proliferative activity. Of 54 analysed, 36 (66.66%) cases had an abnormal karyotyp, while no chromosomal abnormalities could be diagnosed in 18 (33.33%) cases. Of 36 abnormal karyotypes, predominant abnormality were autosomal trisomies in 22 (61.11%) cases, including various chromosome groups (B,C;D,E,G), most of them represent nonviable defects. Follows 6 (16.65%) polyploidies and 4 (11.11%) X monosomies (46,XO). Relatively rare type of double abnormality in 2 cases (5.55%) (48,XY;D14+,E17+; 48XX;C7+,D13+) were observed. Mosaicism (46,XY/47,XY) was present in one case (2.77%) where autosomal trisomy could not be determined. Translocation (46,XY)t(D13,D14) was also present only in one (2.77%) case.

**Conclusion:** Althought CVS analysis in our study did not exclud normal karyotype, our results suggest that missed abortion is strongly associated with chromosome abnormalities. The factors responsible for missed abortion with a normal karyotype are presently unknown and require further study.

**P03.51****MLPA validation for prenatal diagnosis of chromosomal anomalies: retrospective study of 125 consecutive chorion villi samples**

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Rapid screening for common aneuploidies (trisomy 13, 18, 21 and those involving sexual chromosomes) by interphase nuclei FISH or multiplex QF-PCR has become a common offer in prenatal diagnosis. Although it reduces maternal anxiety by discarding the most frequent chromosomal anomalies, some other like terminal deletions are missed. In addition, rapid detection of unbalanced products of parental balanced translocations requires the use of additional specific FISH probes. In contrast, MLPA technique with subtelomeric probe-mixes provides information about copy number changes of every chromosome in a rapid (24 h) and simple protocol requiring few material. Although specific probe-mixes for the detection of common anomalies in prenatal diagnosis have been validated, the aim of the present study was to evaluate the use of subtelomeric probe-mixes for the rapid detection of aneuploidy, unbalanced reciprocal translocations and terminal chromosomal rearrangements of every chromosome on a hundred and twenty-five consecutive *chorion villi* samples. Terminal deletion or duplication was suspected when two different probes for a chromosomal arm appeared deleted or duplicated, respectively. Aneuploidy was suspected if probes for both arms of a chromosome appear deleted (monosomy) or duplicated (trisomy). Results demonstrate the capability of MLPA on detecting not only numerical but also structural chromosomal anomalies on prenatal samples. Thus, this technique can also be a rapid and simple approach for the detection of balanced or unbalanced products of translocations.

**P03.52****22p derived supernumerary marker chromosome identified prenatally by MLPA**

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Supernumerary Marker Chromosomes (SMC) have a frequency of around 0.05% in prenatal diagnosis. Risk for congenital anomalies ranges from 7% for SMC derived from acrocentric chromosomes to 28% for SMC derived from autosomes.

We present a prenatally detected SMC in a 32 years old woman, with a previous spontaneous miscarriage. The ultrasound performed at 11<sup>th</sup> gestational week detected a cystic hygroma. Chorionic villus sampling was done at that time and FISH for 13, 18, 21, XY chromosomes (Aneuvysin, Vysis) was normal. No foetal karyotype could be obtained, and despite normal ultrasounds during follow up, an amniocentesis was performed at the 16<sup>th</sup> gestational week. The karyotype of the fetus was 47,XY+mar. The marker chromosome was centromeric, satellite and smaller than chromosome 21. Because of the risk of Congenital Heart Disease, we performed a FISH with TUPLE1 probe (Catch 22) (Vysis) that was normal. Parental karyotypes were also normal. In order to identify the origin of the SMC, we performed Multiplex Ligation Probe Amplification (MLPA) with Salsa P070 for Telomeres (MRC-Holland). The result was compatible with partial trisomy of 22p. Considering the result of FISH and MLPA, we conclude that the breakpoint lied between the end of IL17R and the beginning of HIRA, carrying some or all the genes associated with Cat Eye Syndrome. The parents decided to terminate the gestation.

This case illustrates the difficulties in prenatal genetic counselling when a SMC is involved and the need to use all technologies available to better predict the outcomes of these pregnancies.

**P03.53****Cell selection in culture of amniotic fluid cells: report of three cases with low level of true mosaicism**

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**<sup>1</sup>Hospital Clínic de Barcelona, Barcelona, Spain, <sup>2</sup>CIBERER, Barcelona, Spain.** Mosaic aneuploidy is found in 0.3% of all amniocentesis. When detected in prenatal diagnosis is a troublesome problem on interpretation and genetic counselling. We present three cases with low level of mosaicism:

1.- Twenty cells analysed from two independent vessels showed 47,XXX, which is a good prognostic result. But a culture performed in another laboratory showed a 45,X/47,XXX with a 75% of monosomic cells. We revised our slides and found 7/40 of 45,X cells, which changed the genetic counselling.

2.- The first culture showed 1/50 of 47,XY,+21 cells, the second and the third showed 5/50 and 2/25 trisomic cells, respectively. This was an ambiguous result, with 6% of +21 cells. We performed QF-PCR analysis for common aneuploidies with a portion of fresh amniotic fluid and the result was compatible with trisomy 21.

3: The cell culture in this case was slow and poor, in order to ensure the diagnosis, we decided to perform QF-PCR. The result was a normal male. Finally, we could obtain some metaphases for cytogenetic analysis and the result was 37 cells with a 45,X karyotype. The gestation was interrupted because of ultrasound findings. We repeated cytogenetics, FISH and QF-PCR studies and the result was 45,X/46,XY. Conclusions: There is a selection in the culture that may disturb the real percentage of abnormal cells. Then, the percentage of abnormal cells does not reflect the state of the fetus. The use of complementary techniques such as QF-PCR and FISH may help to the final result.

**P03.54****Identification of feto-maternal differentially methylated regions on chromosome 18 and chromosome 21. Towards the development of fetal epigenetic markers for trisomies 18 and 21**

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The discovery of free fetal DNA in maternal plasma has raised the possibility for future development of noninvasive prenatal diagnosis (NIPD). We have been using Methylated DNA Immunoprecipitation (MeDIP) assay and DNA oligonucleotide array technology to screen for differentially methylated sequences between maternal blood and placental (fetal) DNA that could be exploited for NIPD. The oligo arrays used are specific for chromosome 18 and chromosome 21. The probes are 50-60bp long with a median spacing of 170bp for chromosome 18 and 70bp for chromosome 21.

The results have shown regions of several consecutive oligo array probes to be differentially methylated between whole blood and placenta. A detailed analysis has revealed 42 regions on chromosome 18 and 34 regions on chromosome 21 that can potentially be used as fetal DNA epigenetic markers. The methylation status of the SERB1NB5 promoter region and two additional regions on chromosome 18 as well as ten regions on chromosome 21 have been confirmed by real time quantitative PCR in six whole blood and six placental DNA samples. Furthermore a high degree of variability has been observed between first and third trimester placentas whereas a lower degree of variability has been detected between individual samples of the same gestational age. Additionally, to imitate the low percentage of fetal DNA observed in the plasma of pregnant women, we have prepared mixtures of 5% fetal DNA with maternal DNA and tested the enrichment of fetal DNA by using MeDIP. Preliminary results have shown enrichment of fetal specific methylated DNA sequences absent in whole blood DNA samples.

**P03.55****Partial trisomy 3q and monosomy 15q due to paternal t(3;15)(q26.33;q26.1); paternal detection by m-CGH and FISH, prenatal USG, postnatal and autopsy findings**

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A 31 years old gravida was referred at 13 week of pregnancy for prenatal diagnosis due to previous four undiagnosed pregnancy loss. During non-invasive screening at 14 week of pregnancy cystic hygroma (1.33 cm), features of cardiac insufficiency, multiply bone anomalies and prune belly syndrome suspicion were noted. Urgent karyotype analyses of both parents with resolution 450 - 650 bb. not detected any anomalies. Amniocentesis (with suspicion of X's chromosomes anomaly) was performed at 15 week and didn't detect any pathologies in classical cytogenetics analyses, but mCGH investigation (2,44 OLI-GO m-CGH Agilent) showed deletion in 46XY foetus chromosomes of distal 15q26.1qter and duplication of 3q26.33qter, confirmed by FISH with use of telomeric probes (Tel Vision 3qSO and 15qSO) and described as 46XY, ish der (15)t(3;15)(qter+,qter-)pat. Aberration was result due to next detected paternal balanced subtelomeric translocation 46,XY,ish t(3;15)(qter-qter+;qter+qter-). In next USG observations Intrauterine Growth Retardation, hypoplastic long bones, feet and hands anomalies, heart defect in form of CoA, hypoplastic DA, VSD, polycystic kidneys and defect of abdominal muscle were diagnosed. After uneventful pregnancy a boy (2130g, 40 cm, Apgar 6,6,7) was born preterm at 36 week. Due to their poor status (particularly renal anomalies and insufficiency) possibility of cardiosurgery correction was excluded and he died at 40 day of life. Autopsy confirmed and précisied all detected in foetal USG anomalies and more like: in the heart aorta-truncus pulmonalis anastomoses, additional perimembranous VSD, bilateral hydronephrosis with urethral and urachal hypoplasia and polysplenia.

#### P03.56

#### Total cell free and cell free fetal DNA quantification in preeclamptic pregnant women

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**Background:** Presence of cell free fetal DNA in plasma of pregnant women is a well known phenomenon. The quantity of DNA is different in normal and pathological pregnancies. The aim of our study was to measure and to compare the quantity of total free and fetal origin DNA in the plasma of preeclamptic patients, and patient with normal pregnancy and to reveal the correlations with pregnancy age and body mass index (BMI) in both groups. **Methods:** Blood samples were collected, and plasma was separated from 71 preeclamptic and 71 patients without symptoms of preeclampsia. Quantitative real-time PCR analysis of the SRY region of Y chromosome and globin gene was performed in order to detect and to measure the quantity of cell free fetal DNA and total free DNA in plasma. **Results:** The mean pregnancy age in preeclamptic and control group was 37 and 34 weeks respectively, BMI ranges between 20.6-38.2 and 16.7-30. The mean value  $\pm$  SD, of total free DNA was:  $6.16E-03 \pm 0.23E-03$  ng/mL and  $2.755E-03 \pm 0.32E-03$  ng/mL, in SRY positive cases the quantity of free fetal DNA we measured  $3.363E-04 \pm 1.28E-05$  ng/mL and  $1.04E-04 \pm 0.92E-05$  ng/mL respectively. The difference between two groups in both cases was significant ( $P=0.001$ ). **Conclusions:** The quantity of free DNA is significantly higher in preeclamptic cases than in patients with normal pregnancy and depends on maternal weight. In concordance with other studies the quantitative measurement of total cell free and cell free fetal DNA could be a predictive marker in early diagnosis and prevention of preeclampsia.

#### P03.57

#### Reproductive decision of Spanish families with genetic risk for peroxisomal diseases

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Peroxisomal disorders are severe neurodegenerative diseases caused by defects in genes that control single steps of metabolic peroxisomal pathways as well as the proteins involved in the peroxisomal biogenesis. Since 1988 we have diagnosed 198 cases of Peroxisomal dis-

eases, 158 of them had been X-linked adrenoleukodystrophy (X-ALD), 2 other isolated defects of peroxisomal β-oxidation and 38 defects of peroxisomal biogenesis (DPB).

The 158 affected X-ALD/AMN males proceed from 95 families that requested prenatal diagnosis (PD) in 34 pregnancies. In all the affected foetuses, parents opted by termination. Couples that were against this option decided not request PD. In most cases the mother was a carrier detected in the course of family studies, who proves the relevance of the recommendation to the parents of the index cases to communicate the genetic risk to other family members. As far as we know, only in one case, the couple hide the information to the family, resulting later on in the birth of one affected child. A couple that undertake 4 pregnancies with the result of 4 affected male foetuses, requested preimplantatory sex selection, but they don't succeed in two consecutive cycles of in vitro fertilization.

Families with other Peroxisomal disorders asked for DP in 11 pregnancies, 4 at risk for an isolated defect of peroxisomal β-oxidation and 7 at risk for PDB.

#### P03.58

#### A case of de novo 16p rearrangement diagnosed prenatally

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A case of a terminated pregnancy of a 30-year-old woman is reported in which ultrasound examination at 22 weeks' gestation showed two umbilical cord vessels, enlarged polycystic right kidney, implying polycystic kidney disease, and a normal left kidney. Conventional chromosome analysis (GTG banding) revealed addition of chromosomal material to the long arm of chromosome 16; however the limited resolution of conventional prenatal karyotype analysis prevented the identification of the origin of the additional material. MLPA (Multiple Ligation-dependent Probe Amplification) analysis was performed, which showed neither duplication nor deletion in chromosome 16. Both parents were found to have a normal karyotype. Expected data will include Fluorescence In Situ Hybridization (FISH) analysis to identify the chromosomal origin of the additional material. Furthermore, array CGH analysis will be performed to define the chromosomal breakpoints and the size of the additional material.

#### P03.59

#### Polyplioidy in early spontaneous abortions

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Polyplioidy is a condition in which there is more than two sets of chromosomes. A total of 321 cases of first trimester spontaneous abortions between 4 and 13 weeks of gestation were analyzed cytogenetically by direct - preparation method using chorionic villi. Among 54% of abnormal karyotypes, trisomy was predominant. The second most common abnormality was triploidy found in 25 (7,8%) cases. Triploidy may arise from fertilization of haploid egg by two haploid sperm or by maternal or paternal meiotic errors. Tetraploidy is a rare ploidy abnormality and was detected in 3 (0,9%) cases, 92,XXYY and 92,XXXX sex chromosome complement.

Among the triploid abortions the gonosomal constitution of XXY prevailed (14 cases), followed by XXX (8 cases) and XYY (3 cases). The maternal age ranged from 18 to 35 age and the gestational age from 6 to 13 weeks. In this study the frequency of poliploidy abortions decreased with maternal age, what confirms that increased maternal age is not a risk factor and mechanism of poliploidy.

Triploidy and tetraploidy together account for 18% of chromosomal abnormalities and give rise to a significant proportion of human pregnancy wastage. Poliploidies are numerical abnormalities, are sporadic, and they do not usually recur in subsequent pregnancies.

**P03.60****The results of perinatal testing from Istanbul Memorial Hospital**

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## Introduction

The aim of this study is the retrospective data collection of patients (especially PGD patients) between 2000-2007 for amniocentesis and for CVS between 2003-2007 to understand that the PGD might be a reasonable option for translocation carriers, advanced maternal age (ama), single gene disorder (sgd) carriers, pathological USG findings, recurrent implantation failure (rif), recurrent pregnancy lost (rpl), bad obstetric history.

Mat- method: Cell cultures were used for amniotic fluid specimens and tissue culture for coryon villi sampling (CVS). Culture developments were observed and underwent harvesting steps in the optimum timing (after 10-12 days). Slides were stained with giemsa staining techniques (GTG) and 30 cells were counted.

Between years 2000-2007, amniocentesis were performed to 615 patients and 82 CVS between 2003-2007 in our center. The indications and chromosome analysis results are in the table.1 below.

## Results:

Table1 Results of amniocentesis

PGT (n=91)	FISH (n=55)	Normal or balanced (n=54)	
		Abnormal(n=1)	mosaic 47,XXY
			Normal or balanced
IVF(n=146)	Single gene disorder(n=36)	Normal (n=36)	-
	Normal (n=141)	-	-
	Abnormal (n=5)	Patologic USG(n=3)	Trisomy 18 Mosaic X 46,-;t(1;2)(p36.1;q12),del(2)(q12q13)
		Maternal age (n=1)	Trisomy 18
		Translocation (n=1)	46,-;dic(8),t(8;18)(q13;q11.1)
Spontaneous (n=378)	Normal(n=361)	-	-
		Patologic USG(n=7)	Trisomy21(n=4) Triploidy 45,X 46,-;t(4;6),t(5;6),t(11;18),der(9)add(9)
	Abnormal (n=17)	Abnormal triple test (n=2)	Trisomy21(n=2)
		Translocation (n=4)	46,-;t(2;7) 46,-;recp t(13;16) 46,-;t(10;13) inv(10)
		Male factor (n=1)	Mosaic X
		Maternal age (n=3)	Trisomy 18 Triploidy Mosaic XXY

Table.2 Results of CVS

Indications	Chromosome Analysis Results	
AMA (n=8)	Normal (n=8)	
Abnormal First Trimester Test(n=30)	Normal (n=29)	Trisomy 21
	Normal (n=10)	
	Trisomy 18	
	Trisomy 21	
Patological USG Findings (n=16)	Mosaic X	
	45,X	
	46,-;t(3;11),t(5;14)	
	47,-;der(15)t(4;15)	
Bad Obstetric History (n=5)	Normal (n=5)	
Male Factor (n=2)	Normal (n=1)	
	46,-;inv(12)	
Abnormal Triple Test (n=2)	Normal (n=2)	
PGD Cases (n=12)	Single gene +HLA(n=9)	Normal (n=9)
	PGD FISH(n=3)	Normal (n=2)
		46,-;t(10;12)

Discussion: In this study, we present that performing amniocentesis and CVS cultures

and karyotype analysis approve that PGD is a reliable option for the patients with several indications to have a non-affected offspring.

**P03.61****Pathological cytogenetical findings in fetal prenatal diagnosis**

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In Medical Genetic Centre in Novi Sad (Institute for Children and Youth Health Care Vojvodina), prenatal genetic screening of fetal abnormalities is performed using detailed analysis of pedigree, maternal and paternal age, biochemical screening (pregnancy associated plasma protein -PAPP-A, free beta-subunit of human chorionic gonadotropin -free beta hCG in 11-14th weeks of gestation; alpha-fetoprotein -AFP, unconjugated estriol -uE3, PAPP-A, in 16-18th weeks of gestation) and expert ultrasound. If married couple have risk for fetal chromosomal abnormalities, Clinical genetics indicate invasive prenatal diagnosis. During last five years (2003-2007.) in Medical Genetics Centre in Novi Sad were made 19019 Genetic consultations, and 11488 invasive prenatal procedures (9938 amniocentesis, 1550 cordocentesis). We found 169 pathological findings (1.47%), from amniocentesis (N=145/9938; 1.46%) and from cordocentesis (N=24/1550; 1.54%). The most frequent chromosomal anomalies was Down syndrome (42.01%), Edwards syndrome (9.46%), Patau syndrome (5.32%), Turner syndrome (6.51%), Klinefelter syndrome (9.46%), and other chromosomal anomalies (27.24%).

**P03.62****Karyotyping vs MLPA in spontaneous (late) pregnancy losses.**

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Early spontaneous abortions (less than 16 weeks of pregnancy) have a variety of chromosome aberrations, most of them aneuploidies of whole chromosomes. Chromosome abnormalities occur to a lesser extent in second and third trimester pregnancy losses. Chromosome analysis provides valuable information about the possible cause of fetal death and the recurrence risk. Yet it is hampered by a high rate of culture failure. Multiplex Ligation dependent Probe Amplification (MLPA) overcomes the problem of culture failure if DNA is extracted directly out of the fetal tissue before culture. Using a subtelomere dedicated test, it can provide information on the ploidy status of all chromosome (arm)s.

In the present study we compared routine chromosome with MLPA analysis in a series of more than hundred fetal tissue samples of spontaneous (late) second and third trimester pregnancy losses. Routine chromosome analysis succeeded in 20% of cases, of which 8% had an abnormal chromosome complement. For MLPA, two different subtelomere specific tests (P036 and P070) were used to examine all chromosomes for ploidy status. We obtained interpretable results in 83% of cases. Abnormal ratio spreads were observed in 6% of them. Detailed data will be presented on the poster.

In conclusion, MLPA increases the success rate for chromosome analysis dramatically by overcoming the problem of culture failure. Although it is suggested that culture failure could be caused by the fact that the fetus has a chromosome abnormality, the percentage of genetically abnormal tissue samples remains similar for both techniques, MLPA and karyotyping.

**P03.63****Prenatal diagnosis of mosaicism for different structural unbalanced cell lines involving chromosome 18**

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Confined placental mosaicism is detected in 1-2% of pregnancies undergoing first-trimester CVS. Structural mosaicism is a rare event, often difficult to interpret. However, chromosome 18 appears to be most frequently involved in these structural rearrangements, according to published cases. We report a further case detected by prenatal diagnosis. CVS was performed on a pregnant woman due to increased

nuchal translucency. Cytogenetic analysis after short-term culture showed four different abnormal cell lines involving chromosome 18, and the karyotype was described as: mos 46,XX,18p+[15]/r(18)[14]/i(18q)[1]/45,XX,-18[3]. Amniocentesis was subsequently performed. Ultrasound examination showed a septate translucency as the only abnormal finding. QF-PCR analysis showed homozygosity for all 18p markers, suggesting a 18p monosomy. The karyotype obtained was 46,XX,del(18p) in all cells. The pregnancy was terminated and pathologic studies are being performed at present. We propose a model to explain the cytogenetic findings. A first postzygotic error, probably an unequal sister chromatid exchange giving rise to a cell line with del(18p) and the complementary with 18p+, may have most likely happened before the first post-zygotic division, since no normal cells have been detected. Isochromosome(18q) and ring(18) seem to be secondary to del(18p), and monosomy 18 may have derived from the ring(18) cell line, given the instability of rings. An unequal distribution of the different cell lines between the trophoblast and the inner cell mass, and differences on the viability of each cell line in different tissues, may explain why the fetal tissues only show the 18p deletion whereas the trophoblast presents the other cell lines.

#### P03.64

#### Uptake of prenatal diagnosis after established carrier status of a balanced structural chromosome abnormality in couples with recurrent miscarriage

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Three to six percent of couples with recurrent miscarriage are carriers of a structural chromosome abnormality. These carriers are offered prenatal diagnosis (PND) in subsequent pregnancies. It is unknown what the effect is of disclosure of the abnormal result of karyotyping in carrier couples on the uptake of PND in subsequent pregnancies. This study evaluates the uptake of PND in carrier couples compared with non-carrier couples.

We collected data of 239 carrier and 389 non-carrier couples presenting for parental karyotyping after two or more miscarriages < 20 weeks of gestation in six centers for Clinical Genetics in the Netherlands, from 1992-2001. PND procedures in subsequent pregnancies were recorded for a minimum of 24 months.

Within the carrier couples 150 out of 239 (63%) underwent a PND-procedure in *at least* one subsequent pregnancy compared to 111 out of 389 non-carrier couples (29%) ( $p<0.01$ ). Only 60 out of 239 carrier couples (25%) underwent a PND procedure in *all* pregnancies.

In the maternal age group < 36 years 136 out of 206 carrier couples (66%) underwent at least one PND procedure.

In the maternal age group  $\geq 36$  years there was no significant difference between carrier (14 out of 33, 42%) and non-carrier couples (47 out of 98, 48%) in the uptake of at least one PND procedure ( $p=0.41$ ).

Detection of a structural chromosome abnormality in couples with recurrent miscarriage has a marked influence on their decision to undergo PND, although one third of all carrier couples refrained from PND in all pregnancies.

#### P03.65

#### Trisomy 13: cytogenetic and prenatal findings

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Objective: To describe prenatal sonographic findings and cytogenetic spectrum of fetuses with trisomy 13 in a tertiary fetal medicine center.

Methods: Retrospective review of cytogenetic laboratory databases identified cases of trisomy 13 from January 1997 to December 2007.

Sonographic and cytogenetic findings were evaluated.

Results: Trisomy 13 was identified in 23 out of 298 cases referred to cytogenetic investigation. Free trisomy was identified in most cases ( $n=20$ [87%]) and chromosome structural abnormalities were identified in 3 cases [13%] of 47XY+13,der(13;14)(q10;q10). Although in 2 fetuses one isolated anomaly was detected, twenty-one cases were found

to carry multiple anomalies. The most frequent structural abnormalities detected were facial anomalies (91.3%[n=21]), holoprosencephaly (60.9% [n=14]) and heart defects (56.5%[n=13]). Abnormal amniotic fluid volume was present in 5 cases. Common findings included urinary obstruction (n=10[43.5%]), microcephaly (n=7[30.4%]) and polydactyly (n=7[30.4%]). Following anomalies were verified in four cases (17.4%): cerebral ventriculomegaly, omphalocele, distended bladder, single umbilical artery and clubbed feet. Other findings: 3 cases of abnormal hands, abnormal foot positioning, echodense kidneys and agenesis of nasal bones; two cases of genital defects and agenesis of one ear; one case of ascitis, edema of the scalp, pleural effusion, ectopic kidney, atypical cranial ossification, bowel dilatation and abdominal mass.

Conclusion: Prenatal diagnosis of trisomy 13 may provide options regarding pregnancy management. Recent advances in sonographic imaging may lead to a frequent update of the findings, without invalidating the applicability of prior reports. Thus, this updated information can be useful for trisomy 13 prenatal characterization and diagnosis.

#### P03.66

#### Rare case of structural aberration of chromosome 18

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Trisomy 18 - Edwards syndrome is the second most common aneuploidy in newborns, with a prevalence between 1 in 3,000 to 1 in 8,000. More than 80 % of cases are caused by free trisomy of chromosome 18, whereas the rest of cases are translocations and other structural aberrations. Subject of this presentation is prenatal capture of rare and special chromosomal constitution of trisomy 18.

Due to positive biochemical screening (routine biochemical screening within the second trimester of gestation) in 17<sup>th</sup> week of gestation, the amniocentesis and karyotype determination have been indicated in 24-year-old mother. Conventional karyotyping of amniotic fluid cells revealed the presence of pseudoisodicentric 18 chromosome, karyotype: 46, XY, psu idic (18)(p11.32). According to recent literature records the breakpoint at 18p11.32 has not been described yet.

Based on result of investigation, parents have decided to terminate the pregnancy in 20<sup>th</sup> week of gestation and it was fully genetically indicated. Karyotype determination of parents has been performed with normal result. It indicates to *de novo* origin of this aberration in fetus. Aborted fetus underwent bioptic investigation. Result of the biopsy confirmed diagnosis of Edwards syndrome. Nevertheless, comparison of proband's phenotypic features with clinical manifestation of free chromosome 18 trisomy is very interesting. Some of the symptoms, present almost in all trisomy 18 cases, have not been found. Most obvious is the lack of clenched hands with crossed fingers and missing congenital heart defect, which are already present during intrauterine development.

#### P03.67

#### Contribution to prenatal genetic counselling in trisomy 20 mosaicism: two new cases

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Trisomy 20 is one of the common mosaic trisomies detected in amniocentesis and it is associated with normal outcome in over than 90% of cases. However, an abnormal outcome has been observed in 5-10% of cases including fetal demise, intrauterine growth restriction and multiple congenital anomalies.

To contribute in defining fetal prognosis , we report two new cases of trisomy 20 mosaicism detected in amniocentesis requested for maternal age.

Following genetic counselling concerning fetal prognosis and on the basis of normal second level US performed at 20 weeks, couples chose to continue pregnancies without proceeding with further invasive controls.

Two healthy babies were born.

To date, follow-up failed to reveal abnormality on physical or psychomotor development at 1 month or at 1,12,18 months, respectively.

#### P03.68

#### Prenatally detected trisomy 7 mosaicism in a child with Blaschko linear skin pigmentary variation and developmental delay.

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We report on a girl showing Blaschko linear skin pigmentary variation associated with developmental delay and minimal facial dimorphisms. The proband's mother was referred for prenatal diagnosis at week 15 of gestation, due to nuchal translucency of 3.5 mm and unique umbilical artery. A chromosomal analysis was performed in cultured amniocytes in two consecutive studies, both showing 46, XX [40%] / 47, XX,+7 [60%]. A study in fetal blood lymphocytes excluded uniparental disomy 7 by microsatellites analysis, and revealed a normal karyotype (46 XX). During pregnancy only intrauterine growth delay was noted. At 45 days of age, the patient was evaluated not disclosing any clinical anomaly and the chromosomal analyses were repeated revealing the previous results. The proband was periodically evaluated showing a progressive developmental delay. In the last review, at 22 months of age, Blaschko linear skin pigmentation anomalies were detected in both lower extremities and thorax associated with sparse hair, abnormal dentition and a moderate developmental delay. This brings up the possibility that the mosaic trisomy 7 line should still be present in skin cells and it could be present in any other tissue.

When a mosaic trisomy 7 is diagnosed prenatally in cultured amniocytes, and it is not confirmed in a fetal lymphocyte karyotype, it is essential to follow up these patients postnatally to establish an early physical, occupational and language therapy in order to improve their developmental outcome. Absence of pigmentary alterations along Blaschko lines at birth is not exclusive, as they could appear any time during childhood.

#### P03.69

#### Foetal ultrasound findings in trisomy 18

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Among liveborn children, trisomy 18 is the second most common autosomal trisomy after trisomy 21. The disorder/condition is characterised by severe psychomotor and growth retardation, microcephaly, microphthalmia, malformed ears, micrognathia or retrognathia, microstomia, distinctively clenched fingers, and other congenital malformations. Approximately 95% of conceptuses with trisomy 18 die in embryonic or foetal life; 5-10% affected children survive beyond the first year of life. The aim of this study was to estimate the correlation between pathological foetal ultrasound findings and trisomy 18.

Over the past five years (2003-2008) we analyzed fetal blood samples for chromosome abnormalities. Samples were taken by cordocentesis and processed using standard techniques. All specimens were G-banded using trypsin-Giemsa. Sixteen metaphase cells were analyzed for chromosomal constitution in each sample.

From 1248 samples of fetal blood analyzed for chromosomal abnormalities, there were 11 (0,88%) with complete trisomy 18. We found no mosaicism, or partial trisomy 18. From 11 fetuses with trisomy 18, 9 (81,8%) had some anomaly detected by ultrasound. Ultrasound findings were polyhydramnion, IUGR, oligohydramnion, heart defect, hydrocephalus and omphalocele.

In the last 5 years in our laboratory, the incidence of trisomy 18 was 0,9%. In all cases, trisomy 18 was complete. About 81% of the fetuses with trisomy 18 had ultrasonographically detected anomalies.

#### P03.70

#### STRs multiplex assay for rapid and economic detection of UPD 15

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Uniparental disomy (UPD) describes the inheritance of both homologues of a pair of chromosomes from only one parent. The clinical impact of UPD and associated imprinting disorders, such as Prader-Willi syndrome (PWS) and Angelman syndrome (AS) increasingly have come to our attention. Chromosome 15 UPD testing is also relevant in various prenatal diagnostic conditions including apparent confined placental mosaicism (CPM), homologous and nonhomologous Robertsonian translocations involving chromosome 15 and 14, and as genomic biomarker for detecting chromosome origin. We developed and validated a two fluorescent STR multiplex assays for a rapid and economic detection of UPD 15 by capillary electrophoresis. Eight informative markers have been selected on the basis of their expected heterozygosity and chromosomal localization. We developed two different four-plex PCRs: multiplex 1 containing microsatellites D15S1007, D15S205, D15S1019 and D15S130; multiplex 2 containing markers D15S988, D15S979, D15S128 and D15S131. In order to assess and validate the polymorphic information content (PIC), allele frequencies and heterozygosity of selected markers we typed 100 unrelated individuals. Statistical analysis revealed a probability to obtain an informative assay of 99.1% for multiplex 1 and 98.8% for multiplex 2. Genotyping results of the two four-plexes were compared with those generated in singleplex reactions for the same samples revealing a concordance rate of 100%. To summarize, UPD exclusion/confirmation is expected to be needed in an increasing number of cytogenetic and clinical cases and we believe that the availability of a rapid test for its recognition should be highly suitable by both genetic laboratories.

#### P03.71

#### Mild/borderline ventriculomegaly as a marker for structural chromosome abnormalities

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Ventriculomegaly is a descriptive term for enlargement of the intracranial ventricular system. It is defined as a ventricular atrium at any gestation, which measures 11 mm or greater. The frequency of chromosomal anomalies reported in the literature in fetuses with ventriculomegaly ranges from 3 to 27%. Separate analysis for mild and moderate ventriculomegaly is not carried out.

We report two prenatal cases with mild ventriculomegaly and structural chromosome abnormalities.

Case 1: prenatal ultrasound at 21 weeks of gestation noted the fetus to have mild ventriculomegaly (V<sub>h</sub>posterior = 11 mm). Chromosome analysis following amniocentesis demonstrated a 1p36 deletion, which was confirmed by fluorescence in situ hybridization (FISH). Subsequent analysis revealed an unbalanced translocation between 1p and 10q resulting in 1p36.1->pter deletion and 10q26.3->qter duplication. Case 2: at 21 weeks of gestation mild ventriculomegaly (V<sub>h</sub>posterior = 11 mm) and intracardiac hyperechogenic focus were detected. Cytogenetic analysis of cultured amniocytes revealed an inverted duplication of the short arm of chromosome 9. The karyotype was: 46,XY,dup(9)(p13p24). In both cases parents opted for termination of pregnancy.

We conclude that borderline ventriculomegaly may occur in association with structural chromosomal abnormalities, and propose that karyotype is offered and FISH analysis is done where a cryptic rearrangement is suspected following classical G-banding techniques.

#### P03.72

#### Prenatal diagnosis of restrictive dermopathy

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Restrictive dermopathy (OMIM 275210) is a rare lethal autosomal recessive disorder characterized by rigid skin, facial dysmorphism (hypertelorism, small nose, open mouth), multiple joint contractures, skele-

etal anomalies (thin ribs, hypoplastic clavicles) and lung hypoplasia. We report prenatal ultrasound findings and molecular genetic diagnosis of this disorder in a woman whose first child died a few hours after birth. From clinical appearance restrictive dermopathy was suspected. There was distant consanguinity of the parents.

Ultrasound examinations in the 29<sup>th</sup> week of a further pregnancy showed growth retardation, short extremities, reduced fetal movements and abnormal shrunken amnion membranes. Additional findings of absent clavicles, thin ribs, an abnormal face with small nose and open mouth strongly suggested the diagnosis of restrictive dermopathy. The shrunken and separated amnion membranes seem to be recorded for the first time in restrictive dermopathy. The child was born in the 32<sup>nd</sup> week of gestation after premature rupture of membranes and died 30 minutes after birth. It showed the expected facial anomalies, a very tight translucent skin with venal pattern and contractures. Morphology of the skin revealed typical findings in restrictive dermopathy with absent rete ridges of the epidermis, hypoplastic appendage structures, very thin dermis and rarefied elastic fibres.

The assumed diagnosis of restrictive dermopathy was confirmed by molecular genetic analysis. A homozygous mutation c.1085-1086insT in the ZMPSTE24 gene was detected. In the following still ongoing pregnancy early molecular genetic prenatal diagnosis could be offered after chorionic villi biopsy resulting in an unaffected fetus.

#### P03.73

##### **Fraser Syndrome In Two Fetuses: Clinical, Radiological And Pathological Evaluation**

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Fraser syndrome (FS;OMIM 219000) is an extremely rare autosomal recessive disorder characterized by cryptophthalmos, cutanaeous syndactyly, ambiguous genitalia, laryngeal/genitourinary malformations, craniofacial dysmorphism and mental retardation. Here, we present two fetuses with FS diagnosed after termination of pregnancy because of severe fetal abnormalities.

Case1: At the 18th week a cordocentesis was performed to the first pregnancy of a consanguineous 26 year-old father and 21 year-old mother because of intraabdominal ascites, unilateral renal agenesis, and cystic adenomatoid malformation of lung (type3)[CCAML3]. Fetal karyotype was 46,XY. After genetic counselling, parents decided to terminate the pregnancy. Fetus presented bilateral cryptophthalmos, hypertelorism, ear/nose anomalies, tetramelic cutaneous syndactyly, single artery/vein, small penis, and scrotal hypoplasia. Autopsy revealed unilateral right renal agenesis, left renal dysgenesis but no CCAML3.

Case2: Amniocentesis for was performed at 25<sup>+</sup>3 th week of gestation of a 29 year-old father and 26 year-old mother because of ultrasound abnormalities: intraabdominal ascites, bilateral renal agenesis, CCAML3, oligo-anhydramnios, nuchal edema, intracardiac hyperechogenic focus, hyperechogenic bowel. Parents were consanguineous. One previous pregnancy ended spontaneously; another was medically aborted. Karyotype was 46,XX. Parents decided to terminate the pregnancy. Fetus showed facial asymmetry, right sided cryptophthalmos, ear/nose anomalies, tetramelic cutaneous syndactyly, umbilical hernia, anterior abdominal wall defect, immature external genitalia, and anal atresia. X-ray revealed 11 ribs. Autopsy confirmed bilateral renal agenesis; gonads were immature, undifferentiated.

#### P03.74

##### **Outcome of fetuses with central nervous system anomalies detected by ultrasound during pregnancy: clinical, cytogenetic, radiological and pathological data from 18 fetuses**

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Fetal brain anomalies constitute a heterogeneous group of disorders with different prognostic outcome. Affected fetuses mostly come to attention because of ultrasound (US) and often on indication termination of pregnancy, before 24th of gestation, and with parental consent, occurs. Here we report outcome of 18 fetuses with brain malformations initially seen on routine US screening during pregnancy. After termination of pregnancy a thorough examination of the fetus, followed by

imaging (X-ray, MRI) and autopsy was done.

Out of 18 fetuses 11 were with chromosomal abnormalities(61%): der(1) (p36.1;p21,3), mosaic trisomy 8, trisomy 18 (two cases), trisomy 21 (one case), 47,XXX, 45, X, 47,XXY, 49,XXXXY, "de novo" inv(9)(p11q13), and a homozygous inv(6)(p23q23). Seven patients presented monogenic syndromes(39%):skeletal dysplasias(3 cases: Short-Rib Polydactyly Saldino-Noonan Type, Thanotrophic Dysplasia San Diego Type and Jarcho-Levin syndrome), alobar holoprosencephaly(2 cases), iniencephaly(one case), Dandy-Walker syndrome(one case) and cranial teratoma (one case). The fetus with iniencephaly showed also paracentric inversion of "both" chromosomes 6. The parents, both were carriers for inv(6q). Following clinical diagnosis all families received genetic counselling and fetal DNAs were isolated for possible Gene mutation analysis.

We believe that management of fetal malformations is an extensive, heavy "team work" yet the best way of structuring bases for phenotype-genotype correlations.

#### P03.75

##### **Congenital lamellar ichthyosis: TGM1 gene mutation analysis and prenatal diagnosis of a twin pregnancy**

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Autosomal recessive congenital ichthyosis include several subtypes: lamellar ichthyosis, Harlequin ichthyosis and non-bullous congenital ichthyosiform erythroderma. Differential diagnosis of these subtypes can be obtained with strict clinical criteria which is crucial for gene-mutation selection. In this report we present prenatal diagnosis of "Congenital Lamellar Ichthyosis" of a mother with twin pregnancy whose previous child was diagnosed with lamellar ichthyosis.

The previous affected child had a history of erythroderma and ectropion since birth. At referral to our center, he presented dry skin with dark scales over his entire body. Scales were also present over the cutaneous surfaces of flexural accentuation. Hyperkeratotic fissures covering palms and soles of the hands and feet were remarkable. Facial skin under eye level was intact and eyelids exhibited mild ectropion. The patient had no other medical problems, and his growth and development have been normal.

TGM1 gene analysis of the index patient showed homozygous mutation at Exon 5, G278R. Amniocentesis was performed for biamniotic twin pregnancy. TGM1 gene analysis revealed heterozygous TGM1 Exon 5 G278R mutations in both fetuses rendering both fetuses being clinical carriers. Karyotypes of the fetuses were normal. Family decided to continue the pregnancy. At birth twins were normal.

#### P03.76

##### **Smith-Lemli-Opitz Syndrome, Prenatal Biochemical and Molecular Diagnosis**

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Introduction: Smith-Lemli-Opitz Syndrome is an autosomal recessive disorder presented with clinical hallmarks like characteristic facial phenotype, cleft palate, thumb-toe abnormalities and ambiguous genitalia. Prenatal and postnatal diagnosis is possible by the detection of low cholesterol and high 7DHC levels in both serum and amniotic fluid. In this report we present a 40 day-old baby diagnosed SLOS Type 1 and prenatal diagnosis in the family's subsequent pregnancy.

Case Presentation: The case presented with growth retardation, cleft palate, facial dysmorphism, unilateral oligodactyly, unilateral congenital cataract and ambiguous genitalia. Biochemical parameters showed low serum cholesterol and high serum 7DHC levels. The child was diagnosed as SLOS. Due to family's demand, prenatal diagnosis during the subsequent pregnancy was performed; the parents were checked for DHCR7 gene mutations. c.384-IVS5+4del and p.S397L mutations were found to be heterozygous in the mother and father respectively. c.384-IVS5+4del is a previously unidentified mutation for SLOS.

An amniocentesis was performed at 16th week of gestation. Amniotic fluid showed normal levels of 7-Dehydrocholesterol and molecular analysis revealed normal DNA results. Cytogenetic analysis revealed

a 46,XY karyotype.

**Conclusion:** Biochemical analysis of the amniotic fluid through 7DHC levels is a rapid and efficient prenatal diagnosis test for SLOS and is family relieving before a results of molecular analysis is achieved. Both pre- and post-natally, association of cleft palate, ambiguous genitalia and oligodactyly in a patient should prompt the physician to evaluate SLOS.

#### P03.77

#### Partial monosomy of chromosome 8p and partial trisomy of chromosome 7p associated with a Fryns syndrome-like phenotype: Prenatal diagnosis

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We wanted to insist on the importance of a good resolution chromosome analysis for finding subtle chromosomal abnormalities in prenatal diagnosis specially when there are abnormal ultrasound findings. We report a prenatal diagnosis performed following the discovery at ultrasound of a diaphragmatic hernia and a Dandy Walker malformation at 16 weeks of pregnancy.

A descision was made to interrupted the pregnancy and the fetal autopsy revealed signs of a Fryns syndrome that is thought to be inherited in an autosomal recessive way : craniofacial dysmorphism (macrocephaly, short webbed neck, coarse face, flat and broad nasal bridge, anteverted nostrils, hypertelorism, microretrognathia, large mouth with thin lips, low-set ears with posterior angulation), Dandy-Walker malformation, diaphragmatic hernia, small penis with hypospadias.

Cytogenetic analysis on the amniotic fluid showed a partial monosomy 8p associated with a partial trisomy 7p which was initially interpreted as a duplication 8p.

When the parents karyotypes were established, we discovered that the father had a terminal reciprocal translocation between the short arm of chromosome 8 and the short arm of chromosome 7 with karyotype 46,XY,t(7;8)(p21;p23).

The fetus had thus inherited an unbalanced translocation with karyotype 46,XY,der(8)t(7;8)(p21;p23)pat.

Array CGH and FISH analysis are under way in order to characterize the break points.

## PO4. Cancer genetics

#### P04.001

#### The APC gene mutations status in Polish FAP patients

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Familial adenomatous polyposis (FAP) is a genetically conditioned predisposition to occurrence of numerous adenomatous polyps in colon and rectum. The polyps arises in second decade of life and left untreated develop to the tumor. Other extracolonic features may include polyps in the upper parts of the gastroenterological tract, desmoid tumours, ocular lesions, osteomas, dental abnormalities, and malignancies in other organs. FAP incidence is estimated at 1/10,000. FAP arises due to germ line mutations in the adenomatous polyposis coli (APC) gene, which was first described in 1991. The APC gene mutations are studied at the Institute of Human Genetics in Poznan for last 10 years. The Institute of Human Genetics in Poznan cooperates with medical centers from all country, what permitted us to create the DNA bank for Polish FAP patients. Till now samples from 340 families were collected. Seven hundred DNA samples from Polish FAP families were banked so far. The APC gene we screened for mutation in 300 probands. We identified 74 point mutations in 124 FAP families. The detected mutation can be considered characteristic for Polish population due to 34 types of them has not described in other population. Among detected mutations seven occurred in two or to the greater number of families. Occurrence of the APC gene large rearrangements was studied in 95 families. We identified rearrangements in 24 fami-

lies in two cases it were deletion of whole APC gene. This work was founded by the Ministry of Education and Science, Poland, grant number 2PO5A10728

#### P04.002

#### Frequent association of sporadic desmoid tumors with germline APC mutations

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As it is known somatic APC mutations are present in desmoid tumors. However there is no information about the role of germline APC mutations in patients with desmoid tumors and without history of familial adenomatous polyposis (FAP).

We examined 31 patients (17 female and 14 male) with apparently sporadic desmoid tumors and without clinical features or family history of FAP. Peripheral blood lymphocytes from these patients were investigated for the APC gene mutations.

Germline APC mutations were found for 6 of 31 the patients (19%). Four mutations - 1462del5, 1465delAG, 1525insT and R2505Q were found for the first time. The mutation 1465delAG was seen twice. The mutations were located predominantly between 1450 and 1525 codons of the APC gene (4 of 5 mutations).

The age of desmoid diagnosis among patients with the APC mutations was ranged from 1 month to 23 years. Colonoscopy was performed for two patients with germline APC mutations and highest age. There were no polyps in colon of 25 years old female. Another patient, 19 years old female had gastric polyps and no colon polyps. In our sample there were six patients with multiple desmoids tumors and five of them were found with germline APC mutations. One patient with APC mutation had intraabdominal desmoid at age 4 year. Thus for the first time it is shown association of sporadic desmoid tumors with APC germline mutations. The patients with germline APC mutations are characterized by early disease onset and severe desmoid phenotype.

#### P04.003

#### Molecular Analysis of the APC gene in Families Affected by FAP: 2 Novel Mutations

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**Introduction:** Familial adenomatous polyposis (FAP) is an autosomal dominantly inherited susceptibility to colon cancer with high penetrance, characterized by more than 100 adenomatous polyps in the colon and rectum. Additional features may include desmoids tumors, polyps in the upper gastrointestinal tract, osteomas and congenital hypertrophy of the retinal pigment epithelium (CHRPE). APC (Adenomatous Polyposis Coli) gene is mutated in most of these carriers. Presymptomatic genetic diagnosis in this syndrome is obligatory as near 100% of carriers of deleterious mutations will present colon cancer by 40 years. The APC gene has 15 exons and an ORF with 8538 nucleotides which code a protein with 2843 amino acids.

**Materials & Methods:** CSGE on APC was set up and used as screening method to analyze APC alterations. Direct sequencing was used to confirm the presence of mutation or polymorphism.

**Results:** we found deleterious mutations in all patients. Two novel mutations c.3921\_3925del AAAAG in MCR and c.1862C>G in exon 14 of APC were found in 2 families with classical FAP

**Conclusion:** Conformation Sensitive Gel Electrophoresis can be used as a simple, cost-effective and sensitive mutation screening test in complex genes where sequencing as the gold standard of mutation detection techniques is expensive to use in the first line.

#### P04.004

#### 15 years of familial adenomatous polyposis diagnostics in the Czech Republic

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Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome with almost 100 % risk of colorectal cancer. FAP exists in two

forms. The typical FAP is characterized by hundreds to thousands of colorectal adenomatous polyps and by extracolonic manifestations. An attenuated form of FAP (AFAP) is characterized by less than 100 adenomas and later onset of the disease. Incidence of FAP/AFAP in the Czech Republic is 1/ 5000 - 7500.

Since 1993 we have detected presence of mutations in adenomatous polyposis gene (APC) and in the last 5 years also mutations in the base excision repair MUTYH gene in FAP families. The mutation in MUTYH gene leads to MUTYH associated polyposis (MAP). The MAP is an autosomal recessive form of polyposis with manifestation similar to AFAP.

We have examined 280 unrelated patients with a suspicion of FAP/AFAP. By the combination of different molecular genetic tests (DGGE, PTT, MLPA, DHPLC, and sequencing) mutation detection rate was 77 % in classical FAP and 40 % in AFAP for APC gene in case the patients met strict diagnostic criteria. For MUTYH gene was mutation detection rate lower than we supposed. In Czech population we discovered 48 unique APC mutations and 3 large deletion of gene.

If the causal mutation is identified, all carriers of the mutation undergo second genetic consultation and the results of molecular genetic testing are used both when stating the predictive diagnosis and in the clinical management of patients, eventually for the prenatal testing.

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#### P04.005

##### Molecular analysis of the APC and MYH genes in Galician and Catalanian FAP patients: a different spectra of mutations?

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Familial adenomatous polyposis (FAP) is an autosomal dominant-inherited colorectal cancer syndrome caused by germline mutations in the APC gene. Recently, patients with multiple colorectal adenomas and also patients with FAP but without detectable germline APC mutations have been found to carry biallelic mutations in MYH.

We analysed the mutational spectrum of the APC gene in 80 unrelated patients (46 Galician and 34 Catalanian) with classical FAP or multiple polyposis, and we also investigated the contribution of MYH germline mutations in those APC-negative patients.

Germline mutations including large deletions in the APC gene were found in 38% of patients. The two commonest hotspots at codons 1061 and 1309 of the APC gene, account for 15% and 12% respectively of the APC-positive families, but were reported mainly in Catalanian families. Thus, mutation at codon 1061 represents the 23% (5/13) of the Catalanian positive families whereas was not found in 17 APC-positive Galician families. The same trend was observed for the codon 1309 mutation. Similar results had been already observed in a previous study with Galician families and could be due to the genetic isolation of this population. Haplotype analyses of these recurrent mutations should be carried out in Catalanian families to investigate the possibility of a common ancestry.

Twenty percent of the APC-negative patients carried biallelic MYH germline mutation, and the two most frequent mutations reported (p.Y165C and p.G382D) were observed. Therefore, MYH analysis is recommended for all APC-negative families, although MYH cannot explain all cases of FAP.

#### P04.006

##### Frequent identification and familial aggregation of hyperplastic polyposis syndrome in the Spanish region of Navarra

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Background: Hyperplastic polyposis syndrome (HPS) is a rare disorder characterized by multiple hyperplastic polyps (HP), and a limited number of adenomas, serrated adenomas and colorectal cancers. Scarce previous series (1-38 patients) have reported exceptional fa-

miliar aggregation and unknown prevalence with predilection for European population.

Aim: To study the prevalence and characteristics of HPS in our region (600,000 inhabitants).

Methods: Retrospective search of patients from the familial cancer registry, gastroenterology and pathology departments fulfilling WHO criteria, or showing essential traits of HPS.

Results: 12 patients from 9 different families fulfilled WHO criteria with a mean of 39 HP per patient (pp), 75% (9/12) mainly right sided; 3.44 adenomas (pp); 2.33 serrated adenomas (pp), and an incidence of colon cancer of 33% (4/12) (mean age= 41y). Remarkably, in two families HPS seemed to be transmitted as a hereditary condition. In the rest, 60% of the patients had at least one 1<sup>st</sup> degree relative with colon cancer (mean age=56y) and 30% had relatives with >5 HP. Besides, we have characterized 6 extra individuals from 5 different families not fulfilling WHO criteria but who met more than one of the essential characteristic of HPS.

Conclusion: The prominent number of patients with hyperplastic polyposis and the familial aggregation in our series support the hypothesis of a higher prevalence of this condition in our region, suggesting a common genetic background. Furthermore, in specially predisposed populations, a reassessment of WHO criteria is probably needed for the diagnosis of additional cases.

#### P04.007

##### The study of the mutations of BMPR1A and SMAD4 genes in the group of Polish JPS patients

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Juvenile Polyposis Syndrome (JPS, MIM # 174900) is an autosomal dominant disorder, characterized by predisposition to developing juvenile polyps. These polyps are hamartomatous lesions of gastrointestinal tract, especially colon and rectum. The juvenile polyps are typified by markedly expanded lamina propria containing dilated cystic glands, an inflammatory infiltrate and show a normal epithelium with dense stroma. The frequency of Juvenile polyposis syndrome is estimate to occur once in every 100000 newborn. Risk of gastrointestinal malignant transformation in JPS patient is increased and ranges more then 60%. Occurrence of the colon cancer is two fold higher in the upper parts of the gastrointestinal tract. Development of the juvenile polyps is caused by mutation in one of two genes associated with JPS. First of them is bone morphogenetic protein receptor 1A gene (BMPR1A also known as ALK3), which encodes a type I cell surface receptor, a serine/threonine kinase receptor, involved in bone morphogenetic protein signaling pathway (BMP). Second one is SMAD4 gene (also known as MADH4 or DPC4). The protein product of this gene is intracellular mediator of TGFβ superfamily signaling pathway. In result of research we observed four mutations. Using screening methods were found two mutations in the BMPR1A gene and two in the SMAD4 gene. Presented results are preliminary stage study which main aim is to determine the mutation spectrum of the BMPR1A and SMAD4 genes in Polish JPS patients. The study was financed by the Ministry of Education and Science, Poland, grant number 2PO5E02630

#### P04.008

##### Timing and Importance of genomic instability in colorectal tumourigenesis and association with clinicopathological features

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Aims:

- To test the hypothesis that somatic genetic instability is a necessary driver of early colorectal carcinogenesis
- To investigate the sequence of molecular carcinogenic changes.

### Techniques

There is good evidence for the hypothesis that colorectal carcinoma initiation is progression from benign adenoma to carcinoma via a series of sequential somatic genetic and epigenetic changes. However it is not known whether genomic instability is a necessary initiating factor.

We are currently recruiting patients with a known family history of colorectal carcinoma or who are undergoing surveillance colonoscopy for recurrent polyps. These patients attend screening colonoscopy, where polyps are identified and removed. To date, 137 patients have been recruited with a total of 195 polyps.

A pilot study, looking at markers (K-ras, BRAF, MSI, LOH) of genomic instability in 168 polyps in 107 patients demonstrated that this was technically feasible.

We will collect 50 hyperplastic polyps and 300 adenomatous polyps, including 30 serrated adenomas, and 10 polyps from patients with Lynch syndrome.

Isolated crypts will be extracted from polyps and tested for genomic instability using several parameters: chromosomal loss/gain, copy number variation, LoH, MSI and CPG-Island Promoter Methylation. We will also screen for mutations in known target genes.

Results will be compared with normal tissue, to determine when/if genomic instability occurs, and its relationship to other (epi)genetic changes

### Conclusions

We hope to elucidate the earliest drivers of colorectal carcinogenesis: when each form of genomic instability occurs, whether they initiate carcinogenesis and how this relates to family history and clinico-pathological features.

#### P04.009

#### **Microsatellites instability and mismatch repair genes mutations in hereditary nonpolyposis colorectal cancer**

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**Background:** Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant cancer predisposition syndrome caused by germ-line mutations in DNA mismatch repair genes. Therefore diagnosis of genomic deletions of one or more exons of the MSH2 and MLH1 genes and the analyse of microsatellite instability (MSI) from a minimal amount of highly damaged DNA is difficult.

**Methods:** Formalin-fixed and paraffin-embedded tissue samples from 25 HNPCC patients were analysed for microsatellite instability (MSI) using a panel of 7 markers (BAT-25, BAT-26, NR-21, NR-24, MONO-27, Penta C and Penta D) included in a commercial kit. MLPA tests were also performed to screen for major mutation in the mismatch repair genes MLH1 and MSH2.

**Results.** The study revealed a 58% microsatellite instability pattern for the 25 unrelated Romanian HNPCC patients. 5 to 10% of major alterations detected on the MLH1 and MSH2 genes are subject for further characterization.

**Conclusions.** So far, family history, MSI and MMR gene mutation analysis become critical parameters for HNPCC genetic characterization and genetic counseling.

#### P04.010

#### **Microsatellite instability and promotor hypermethylation of MLH1 and MSH2 in Bulgarian patients with sporadic colorectal cancer**

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Inactivation of the genes involved in DNA mismatch repair is associated with microsatellite instability (MSI) and loss of heterozygosity (LOH). Mutations in MLH1 and MSH2 genes are usually the cause of hereditary colorectal cancers (CRC), such as HNPCC, while microsatellite instability in sporadic CRC often results from epigenetic inactivation of *hMLH1* due to DNA promotor methylation.

We examined MSI in 98 patients with sporadic CRC and the methylation status of the *MLH1* and *MSH2* promotor regions in the cases with MSI/LOH. MSI was evaluated in a panel of five microsatellite markers (*BAT26*, *D5S346*, *D18S35*, *D2S123* and *FGA*) and the hypermethylation assessed by methyl-specific PCR (MSP) of bisulfite converted DNA. The PCR products were analyzed on agarose gel electrophoresis, following by direct sequencing.

Our results demonstrated MSI/LOH in 29 patients (29.5%) with sporadic colorectal cancer. Among them the promoter hypermethylation of *MLH1* was observed in 14 (48.3%) of the cases, whereas promoter hypermethylation of *MSH2* was observed only in one case (3.4%). The epigenetic changes of *MLH1* were associated with loss of the protein expression.

**Conclusions:** Widespread promotor methylation of tumor suppressor genes is a common mechanism of gene inactivation during tumorigenesis. Our results suggest that microsatellite instability in Bulgarian patients with sporadic CRC and the epigenetic inactivation of *hMLH1* in association with DNA methylation occurs in similar frequency to relative studies of sporadic colorectal cancer. The promotor hypermethylation of *MSH2*, although a rare event, is also a possible cause of mismatch repair system deficiency in this type of CRC.

#### P04.011

#### **Implication of *MSH6* mutations in colorectal cancer (CRC) patients carrying a monoallelic *MYH* mutation**

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**Background.** CCR risk associated with germline monoallelic *MYH* mutations remains controversial. Nevertheless, a slight increased risk has been suggested. *MYH* and *MSH6* proteins act together during the DNA reparation process. It has been shown that individuals harboring mutations in both genes could have an increased CRC risk.

**Aim.** To evaluate the prevalence of *MSH6* mutations in CRC patients carrying a monoallelic mutation in *MYH*.

**Patients and methods.** We analyzed the prevalence of *MSH6* mutations in a CRC patients cohort with and without a monoallelic *MYH* mutation (group I, n=26 and group II, n=50, respectively), and in another cohort including healthy controls with and without a monoallelic *MYH* mutation (group III, n=21, group IV, n=21, respectively). Patients and controls were recruited from EPICOLON and Hospital Clinic, respectively. Mutational screening was performed by TaqMan, SSCP, DHPLC and sequencing.

**Results.** We detected three germline *MSH6* mutations in group I (11.5%), a missense variant (R635G), a change in 3'UTR region (4098A>C) and a nonsense mutation (Q982X). We did not detect any *MSH6* mutation in groups II and IV, whereas only one was found in group III (4.8%) (M1326G). *MSH6* mutations were more frequently found in CRC patients with a monoallelic *MYH* mutation than in non-carriers (11.5% vs 0%, p=0.037).

**Conclusions.** CRC patients harboring a monoallelic *MYH* mutation may carry more frequently a *MSH6* mutation than patients without them. We could hypothesize that the CRC predisposition associated with a mutation in each gene could add up and confer an increased risk.

**P04.012****A novel PTEN mutation in an Italian family with hyperplastic polyposis of the colon in the context of Cowden Syndrome**

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Clinical manifestations of Cowden Syndrome, which is caused by mutations in the PTEN gene, are extremely variable. Among gastrointestinal manifestations, hamartomatous polyps are the typical lesions, but other polyp types, including hyperplastic, have been reported.

We describe a family in which hyperplastic polyposis was the reason for referral to the genetic clinic and that was found to carry a novel PTEN mutation.

The proband, a 46 year-old man, was referred to the cancer genetic clinic because of the endoscopic finding of colonic polyposis. Histology showed the polyps to be hyperplastic. Moreover, upper gastrointestinal endoscopy detected esophageal acanthosis and papillomatosis and HP-related gastritis. Physical examination revealed the presence of macrocephaly (65cm), obesity and cutaneous papillomas. The mother, 62 year-old, had also been diagnosed with hyperplastic polyposis and with carcinoma ex-adenoma of the left colon, for which had undergone hemicolectomy at age 61. Previously, she had had hysterectomy for uterine fibroids at age 47 and mastectomy for invasive ductal carcinoma of the breast at age 51. Physical examination showed macrocephaly (59cm), obesity, cutaneous papillomas and subcutaneous lipomas.

PTEN mutational analysis in the proband revealed the germline heterozygous mutation 303delA in the exon 5, which was confirmed in the mother. This mutation, which to our knowledge has never been described before, generates a stop at codon 111, producing a truncated protein which loses three functionally relevant domains.

To help clarify the pathogenic mechanism of the mutation, we are now performing molecular studies on pathologic tissues removed from the patients.

**P04.013****Dysregulation of RAS signaling in colorectal carcinomas**

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Half of all colorectal carcinomas (CRC) have dysregulation of the RAS signaling, increasing the cellular proliferative potential and resistance to apoptosis. KRAS is known to be commonly mutated in CRC, while mutational status of the NF1 gene, acting as a negative regulator of RAS signaling, is not known. We analyzed a series of CRC for mutations in KRAS, BRAF, and NF1. NF1 coding region screening was performed by dHPLC (denaturing high performance liquid chromatography), sequencing and MLPA (multiple ligation-dependent probe amplification). KRAS and BRAF were analyzed by sequencing. 40% (26/65) of the samples carried a mutation in KRAS, 22% (14/64) in BRAF, and 13% (3/24) in NF1. We found that 62% (40/65) of the samples had alterations in one or more of the components, meaning they have a dysregulation of the RAS pathway. BRAF and KRAS mutations were mutually exclusive. BRAF mutation was strongly associated with microsatellite instability (MSI), female gender, and proximal location. Among the 24 samples analyzed for mutations in NF1, we found 2 missense and 2 splicing mutations. All NF1 mutations occurred in MSI, proximal, and female-derived tumors with BRAF mutation. In addition, we found that 3 of the samples had duplication of parts or the whole NF1 gene. In conclusion RAS pathway is dysregulated by mutually exclusive mutations of KRAS and BRAF in the majority of CRC. We show that the activity of RAS signaling is likely to be enhanced in more than 10% of the tumors since they harbor mutations in both BRAF and NF1.

**P04.014****Age related differences in molecular profiles of colorectal cancers in patients from the Republic of Macedonia**

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Colorectal cancer (CRC) is considered a disease of elderly, though 2-3% occurs in patients <40 years of age. Published data on the differences between young and elderly patients are quite controversial. Our study aims at comparing the molecular profiles between tumors of younger (age <40y) and older (>40y) patients from the Republic of Macedonia. A total of 343 patients with sporadic (FAP & HNPCC excluded) tumors treated by surgery were enrolled in the study. DNA was isolated from peripheral blood and fresh tumor tissues obtained immediately after surgery, and paired samples were evaluated for the presence of MSI and/or LOH using Real-Time PCR or multiplex qPCR. The evaluation included 23 SNP or STR markers located at 1p, 2p, 2q, 4q, 5q, 8p, 11q, 12q, 17p, 17q, 18p, 18q, 21q from either NCI panel or located in regions harboring genes implicated in CRC pathogenesis. Younger patients comprise 6% of CRC in our population. They are predominantly males (76% vs. 57% of older patients) with tumors in proximal colon (40 vs. 27%) and with MSI-H genotype (23 vs. 9.6%). On the contrary, the frequency of LOH is higher in tumors from older patients: 59 vs. 36% at 18q, 55 vs. 20% at 8p, 42 vs. 25% at 1p and 37 vs. 30% at 5q. In 46% of younger and 30% of older patients neither MSI nor LOH was detected. Our data suggest that CRC pathogenesis evolves through different pathways in patients with early compared to patients with late onset of the disease.

**P04.015****Mutational analysis of the PIK3CA kinase domain in endometrial carcinoma**

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Somatic mutations in PIK3CA, which encodes the p110 $\alpha$  catalytic subunit of phosphatidylinositol 3-kinases (PI3K), have been identified in a wide variety of tumors including endometrial carcinoma (EC). Mutations in PIK3CA cluster to exons 1 (p85), exon 9 (helical domain), and exon 20 (kinase domain) of the gene.

Recently, mutations in exon 20 have been linked to unfavorable clinicopathological characteristics of EC patients such as grade and lymphovascular invasion.

We have determined the frequency of exon 20 PIK3CA mutations among 60 endometrial carcinomas and have related their presence to metastatic disease. Five of the patients had metastasis (to ovary, sigma and parametrium).

We performed direct cycle sequencing of PIK3CA exon 20.

We found 4 missense mutations in 6 tumors (10%). We found c.3140A>G (p.H1047R) in 3 cases. We also found c.3073 A>G (p.T1025A) and c.3145 G>C (p.G1049R). In one case we found a mutation which, to our knowledge, has not been reported previously: c.3074 C>T (p.T1025I).

Two of the patients with exon 20 PIK3CA mutations had metastatic disease (33%), while 3 of 54 (5.5%) patients with no mutations had metastasis ( $p=0.07$ ). The mean age at diagnosis for patients with PIK3CA mutation was 62.8, and for patients without a mutation - 63.3.

Somatic mutations in exon 20 of PIK3CA have been shown to be oncogenic as they lead to an increased PI3K activity. The PI3K regulate signaling pathways such as cell proliferation, survival and adhesion. Our results confirm that PIK3CA alterations may play an important role

for endometrial carcinogenesis and invasion.

#### P04.016

#### Identification of four new pathological mutations in HNPCC patients of the Basque Country

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Hereditary non-polyposis colorectal cancer (HNPCC) is the most common form of hereditary colorectal cancer (CRC), accounting for 1%-5% of cases and is associated with germline mutations in the mismatch repair genes (MMR). We have studied families with HNPCC for three years granted by the Public Health Department of the Basque Country. The aim of this work is to establish whether the proportion of mutation in our population is similar to others previously published. Fifty-five index cases were analysed using heteroduplex sequencing and MLPA. Thirty patients fulfilled the Amsterdam criteria and twenty-five fulfilled the Bethesda criteria. Ninety relatives were also tested. We found in total twenty-three variations (41.8%): eight (14.5%) in MLH1; nine (16.4%) in MSH2; and six (10.9%) in MSH6. Of these variations, eighteen were pathologic mutations, four of them being novel. Furthermore, other three new variations were found, but without aminoacid change. The 43.3% of Amsterdam criteria patients had one pathological mutation and so had 28% of the Bethesda criteria. Of the thirty-nine relatives with mutation, six have only developed cancer. The majority of pathological mutations in Amsterdam criteria were found in the MLH1 and MSH2 genes. In the MSH6 the pathological mutations were found in Bethesda criteria patients, that presented many different types of cancer. In this study we haven't found any rearrangements in the MLH1 gene, as were described in other populations. In conclusion, with this work we are contributing these new mutations to the wide spectrum that alredy exists.

#### P04.017

#### Uncertain pathogenicity of MSH2 variants N127S and G322D challenges their classification

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Hereditary non-polyposis colorectal cancer (HNPCC) is associated with germline mutations in mismatch repair (MMR) genes. Inherited missense mutations, however, complicate the diagnostics since they do not always cause unambiguous predisposition to cancer. This leads to variable and contradictory interpretations of their pathogenicity. Here, we establish evidence for the functionality of the two frequently reported variations, MSH2 N127S and G322D, which have been described both as pathogenic and non-pathogenic in literature and databases. We report the results of three different functional analyses characterizing the biochemical properties of these protein variants in vitro. We applied an immunoprecipitation assay to assess the MSH2-MSH6 interaction, a bandshift assay to study mismatch recognition and binding, and a MMR assay for repair efficiency. None of the experiments provided evidence on reduced functionality of these proteins as compared to wild-type MSH2. Our data demonstrate that MSH2 N127S and G322D per se are not sufficient to trigger MMR deficiency. This together with variable clinical phenotypes in the mutation carriers suggest no or only low cancer risk in vivo.

#### P04.018

#### Familial and clinicopathological differences in Amsterdam-families according to their mismatch repair status

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The aim of this study was to establish the familial and clinicopathological differences within Amsterdam-families according to their mismatch repair (MMR) status.

Seventy families fulfilling Amsterdam criteria II were included. MMR status was determined by microsatellite analysis and/or immunohisto-

chemistry of MMR proteins. Families with microsatellite instability and/or loss of expression of MMR proteins were considered MMR-deficient. Families were classified in three groups: A: MMR mutation detected (Lynch syndrome) (n=36); B: MMR-deficient families with no MMR mutation detected (n=10); and C: MMR-proficient families (n=24).

Proband's age of diagnosis was younger in Lynch syndrome families (A, 40 y; B, 50; C, 46 y; p=0.028). The presence of extracolonic tumors, the absence of polyps and the number of first-degree relatives with HNPCC-associated tumors were higher in Lynch syndrome families. MMR-proficient families showed a predominance of left colon tumors with no mucinous phenotype and a lower presence of metachronous tumors.

These results confirm that the presence of a MMR mutation is associated with an specific phenotype, while MMR-deficient families with no detected mutation show a mixed phenotype likely reflecting misclassification.

#### P04.019

#### Pathogenicity analysis of six unclassified missense variations in MMR genes identified in a selected population of Castilla-León (Spain)

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Lynch syndrome is the most common hereditary syndrome predisposing to colorectal cancer. Mostly, it is caused by DNA variations in the DNA mismatch repair (MMR) genes MLH1, MSH2 and MSH6. Of considerable relevance in the clinical management of colorectal cancer is to know if the missense variants referred to as unclassified variants (UVs) are or not cancer-causing. We report the mutational screening of 100 index cases from the Council Genetics Unit of Castilla-León. Of the 21 (21%) who carried germline mutation, 11 (52%) were categorized as pathogenic (frameshift, large rearrangements, splicing defects) and 10 (48%) as unclassified missense variants. The focus of this report is the classification of six of these missense variants as benign or pathogenic: MLH1 c.1852\_1853AA>GC **p.K618A**; MLH1 c.2146G>A **p.V716M**; MSH6 c.2633 T>C **p.V878A**; MSH6 c.431 G>T **p.S144I** and two novel variations MSH6 c.3425 C>T **p.T1142M**; MLH1 c.1574 G>A **p.S505N**. To test this, we have analyzed clinical and genetic aspects including i) comparison between allelic frequency of the variant in the cases and 700 unaffected controls from a National DNA Register ii) a co-segregation study of the mutation with the disease within pedigree iii) evolutionary conservation of the involved amino-acid and type of aminoacid change iv) a prediction of the degree of affection at the protein function by two bioinformatics programs "Sort intolerant from tolerant" (**SIFT**: <http://blocks.fhcrc.org/sift/SIFT.html>) and Polymorphism Phenotype (**PolyPhen**: [www.bork.embl-heidelberg.de/PolyPhen](http://www.bork.embl-heidelberg.de/PolyPhen)). The combination of all these approaches helps us to predict whether these contribute to the disease phenotype or merely represent rare polymorphisms

#### P04.020

#### A Database to Support the Clinical Interpretation of Human Mismatch Repair Gene Variants

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Germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2 can cause Lynch syndrome. Truncating MMR gene mutations generally offer a clear handle for genetic counselling and allows for pre-symptomatic testing. In contrast, the clinical implications of most missense mutations and small in-frame deletions detected in patients suspected of having Lynch syndrome are unclear. We have constructed an online database dedicated to these unclassified variants (UVs) of the MMR genes. It can be easily searched for information on the results of functional assays and other findings, including tumour characteristics, segregation within families and frequency in controls. The clinical classification of MMR gene UVs will be a huge challenge. Recently formed committees within the International Society for Gastrointestinal Hereditary Tumours (InSiGHT) are working towards collecting data and developing classification algorithms. Our MMR gene missense database will be one of the tools to support that process. It can be searched at [www.MMRmissense.org](http://www.MMRmissense.org)

**P04.021****Comparative scanning of polymorphisms in mitochondrial Dloop region in colorectal and breast cancer**M. Akouchekian<sup>1,2</sup>, M. Houshmand<sup>2,3</sup>;

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**ABSTRACT**

Mitochondrial DNA (mtDNA) mutations have been found in many kinds of human cancer and especially the 1.1 kb displacement loop (D-loop) region was found to be a "hot spot" for mutation in mtDNA of tumors. Mitochondria play an important role in shifting the cell from death to abnormal cell growth thus contributing to the neoplastic process, because of their essential roles in energy metabolism and generation of reactive oxygen species (ROS) also initiation of apoptosis and aging. The purpose of this study is to scan the mutation frequencies in hypervariable regions of mitochondrial D-Loop in colorectal and breast cancer patients comparatively. The results of our investigation are summarized in the following table. When each polymorphism is tested individually using the Fisher exact test the frequency of two single-nucleotide polymorphism SNPs were found to be significantly different between the colorectal cancer (CRC) and breast cancer patient ( $p \leq 0.05$ ). While the SNP C16261T ( $p=0.0005$ ) is significantly more in breast cancer patients than CRC patients, the frequency of SNP T16519C ( $p=0.0019$ ) is less in breast cancer patients than CRC patients.

In this contribution the fact that SNP C16261T may be a consequence for breast cancer and SNP T16519C for CRC is discussed.

Fisher's Exact Test <i>p</i> value	Breast cancer cases (n=6)		Colorectal cancer Cases (n=40)	
	%	Positive	%	Positive
0.0005	83.3	5	10	4
1	16.6	1	25	10
1	0	0	10	4
0.0019	0	0	70	28

**P04.022****Identification of two *MLH1* founder mutations in Spanish Hereditary Nonpolyposis Colorectal Cancer families**

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Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal disorder caused by mutations in DNA mismatch repair genes, most often in *MLH1*, *MSH2* and *MSH6* genes. Tumors of HNPCC-spectrum are associated with microsatellite instability and loss of mismatch repair proteins expression. In some populations, founder mutations appear to explain a substantial fraction of HNPCC cases.

We report here the identification of two mutations in *MLH1* gene, c.306+5G>A and c.1865T>A (p.Leu622His), in 12 and 11 Spanish HNPCC families, respectively. Both mutations segregate with the disease, and tumors show microsatellite instability and loss of expression of *MLH1* protein. RNA studies demonstrate that c.306+5G>A mutation generates two aberrant mRNA transcripts. Experimental approaches are being performed to elucidate the pathogenicity of the c.1865T>A (p.Leu622His) mutation.

Haplotype analysis is performed using three single nucleotide polymorphisms of *MLH1* gene, three intragenic and four extragenic microsatellite markers, located between D3S1609 and D3S3564. We identify two common haplotypes associated to c.306+5G>A and c.1865T>A, suggesting a founder effect.

Mutations c.306+5G>A and c.1865T>A (p.Leu622His) of *MLH1* gene are the first founder *MLH1* mutations identified in Spain. This fact would have important implications for the design of molecular diagnostic strategies in the HNPCC Spanish families.

**P04.023****Further evidence for heritability of an epimutation in one of twelve cases with *MLH1* promoter methylation in blood cells clinically displaying HNPCC**

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**ABSTRACT**

Germline mutations in mismatch repair (MMR) genes, tumours with high microsatellite instability (MSI-H) and loss of MMR protein expression are the hallmarks of HNPCC (Lynch-syndrome). While somatic *MLH1* promoter hypermethylation is generally accepted in the tumorigenesis of sporadic tumours, abnormal *MLH1* promoter methylation in normal body cells is controversially discussed as a mechanism predisposing patients to HNPCC.

94 patients suspected of HNPCC-syndrome with a mean age of onset of 45.5 years, *MLH1*-deficiency in their tumours but no germline mutation underwent methylation-specific PCR-screening for *MLH1* promoter methylation.

In peripheral blood cells of twelve patients an *MLH1* promoter methylation, in seven informative cases allele-specific, was found. Normal colonic tissue, buccal mucosa, and tumour tissue available from three patients also presented abnormal hemiallelic methylation in the *MLH1* promoter. The heredity of aberrant methylation is questionable. Pro: *MLH1* promoter methylation was found in a patient and his mother giving evidence for a familial predisposition for an epimutation in *MLH1*. Contra: a de novo set-up of methylation in one patient, a mosaic or incomplete methylation pattern in six patients, and no evidence for inheritance of *MLH1* promoter methylation in the remaining families. Our findings provide strong evidence that *MLH1* promoter methylation in normal body cells mimics HNPCC and constitutes a pathogenic pre-lesion in *MLH1*. The identification of hypermethylation as an epigenetic defect has important implications for surveillance recommendations, as these patients should be treated like Lynch-Syndrome patients, whereas the heritability of methylation is still under investigation.

**P04.024****Founder effect of a pathogenic *MSH2* mutation identified in four Hereditary Non-Polyposis Colorectal Cancer (HNPCC) families**

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HNPCC is caused by germline mutations in DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*. The novel *MSH2* c.[2635-3T>C; 2635-5C>T] mutation was identified in 4 HNPCC families, cosegregating with the disease. This mutation, located in intron 15, was predicted to alter the correct mRNA processing by *in silico* analysis.

Our aim was to perform the c.[2635-3T>C; 2635-5C>T] mutation screening in HNPCC and control populations, to evaluate the founder effect in our population by haplotype analysis and to confirm the pathogenic effect of the mutation by *MSH2* expression studies.

Mutation screening was performed by SSCP and CSCE in genomic DNA from 256 HNPCC index cases and 239 controls. Haplotyping was performed analysing 4 *MSH2* extragenic microsatellite markers (D2S288, D2S2227, D2S1247 and D2S1248) in 50 controls and mutation carriers by using the PHASE program. We analysed the effect of the mutation in mRNA processing by RT-PCR and in *MSH2* expression by qRT-PCR using RNA from 5 mutation carriers and 18 controls. None of the remaining HNPCC cases or controls analysed harboured the mutation. We identified a common telomeric haplotype and two centromeric haplotypes, both rare in our population. Although we were not able to identify any abnormal transcript by RT-PCR with the design used, we observed a 50% reduction of mRNA *MSH2* expression in carriers when compared with controls. Haplotype analyses suggest a founder effect of the c.[2635-3T>C; 2635-5C>T] *MSH2* mutation and expression studies support a pathogenic role of this mutation.

#### P04.025

##### Biallelic MUTYH germline mutations and endometrial cancer

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MAP (MUTYH-Associated Polyposis) is an autosomal recessive condition predisposing to colorectal cancer, due to inherited defects of the MUTYH gene causing an excess of somatic G>T mutations in the APC and KRAS genes. To date, few extracolonic manifestations have been observed in MAP patients, and the clinical spectrum of this condition is not yet fully established. Recently, one patient with a diagnosis of endometrial cancer and biallelic MUTYH germline mutations has been described (Barnetson et al., Clin Genet 2007; 72: 551-555). However, it is not yet clear if biallelic germline mutations in MUTYH increase the risk of endometrial tumours. We report on two MAP patients with biallelic MUTYH germline mutations who developed endometrial carcinoma. In one of the patients, a diagnosis of Lynch syndrome was originally considered, and subsequently ruled out by immunohistochemical analysis of mismatch repair proteins and microsatellite instability analysis. The endometrial tumours were evaluated for PTEN, PIK3CA, KRAS, BRAF and CTNNB1 mutations. A G>T transversion at codon 12 of the KRAS gene was observed in one tumor. A single 1-bp frameshift deletion of PTEN was observed in the same sample. Overall, these results suggest that endometrial carcinoma may represent a component manifestation of MAP.

#### P04.026

##### Gene conversion rates between PMS2, a mismatch repair gene involved in Lynch syndrome, and its pseudogene PMS2CL; impact on the reliability of current mutation scanning

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Mutation detection in PMS2, a Mismatch Repair gene involved in Lynch syndrome (formerly Hereditary Non Polyposis Colorectal Cancer or HNPCC), is complicated by the presence of the pseudogene PMS2CL. It has been shown that gene conversion can occur between the 3' part of PMS2 and this 700 kb proximally located pseudogene. We investigated 83 familial colorectal cancer cases and 25 healthy controls by semi-quantification of paralogous sequence variants (nucleotides that differ between PMS2 and PMS2CL) in sequence chromatograms of non-specific PCR products (amplification of PMS2 and PMS2CL sequences simultaneously) and show that, from exon 12 onwards, all PMS2 specific nucleotides on which commonly used MLPA and PMS2 specific PCR primers are based, can be subject to gene conversion with an increasing frequency of up to 50 % at the 3' end of the gene. Current mutation scanning designs are thus extremely prone to generate false-negatives or even worse false-positives in this part of the gene.

With a newly developed protocol including non-specific sequencing, cDNA analysis and Southern Blot analysis that excludes false-nega-

tive and false-positive results due to gene conversion events, we scanned colorectal cancer patients including strong candidates for a PMS2 germline mutation based on immunohistochemistry analysis of their tumours. We show that several mutations detected in the 3' part of PMS2 using the current mutation scanning designs are actually false-positives and that also mutations can be missed.

#### P04.027

##### Screening for genomic rearrangements of the *MMR* and *APC* genes in Galician families with hereditary colorectal cancer

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Lynch syndrome and Familial Adenomatous Polyposis (FAP) are two well-known inherited colorectal cancer syndromes caused by germline mutations in susceptibility genes (*MMR*, *APC*, *MYH*). Molecular diagnosis has until very recently relied only on detection of germline point mutations in these genes. Nevertheless, it is known that large rearrangements also play an important role in pathogenesis and should therefore be considered.

Thirty-nine FAP and 16 Lynch syndrome unrelated Galician families that were negative for germline mutations were analysed by MLPA to determine the frequency of large rearrangements. Positive results were further confirmed by other methods (FISH, cDNA analysis).

Three different deletions in *APC* were detected in 4 families (8% of the total *APC* families): an exon 4 deletion, an exon 1-15 deletion, and two whole gene deletions. Carriers of whole allelic deletions presented a severe polyposis phenotype with an early onset of symptoms.

In Lynch syndrome families, 3 deletions were found in *MSH2* (exon 7-16, exon 2-3 and one whole gene deletion), whereas only one deletion was found in *MLH1* (exon 4-6), making up to 14% (10% *MSH2* and 4% *MLH1*) of the Lynch syndrome germline mutations. Large rearrangements were mainly detected in families fulfilling Amsterdam criteria. The overall results resemble those previously published in other populations and confirm that large rearrangements represent an important percentage of FAP and Lynch syndrome germline mutations. They should therefore be taken into account in molecular genetic testing of Galician families.

#### P04.028

##### New germline mutations of the *MLH1* mismatch repair gene in brazilian hereditary non-polyposis colorectal cancer

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**Background:** Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant syndrome predisposing to the early development of various cancers including those of colon, rectum, endometrium, ovary, small bowel, stomach and urinary tract. HNPCC is caused by germline mutations in the DNA mismatch repair genes, mostly *MLH1* and *MSH2*. The mutation spectrum in the Brazilian population is still poorly documented.

**Aim:** To report our experience on the mutation screening and investigation of the pathogenicity of the mutations. in *MLH1* and *MSH2* genes in 20 unrelated Brazilian kindreds with suspected HNPCC.

**Methods:** Twenty brazilian patients with familial clustering of CRC were genetically tested. The families were identified by applying the Amsterdam and Bethesda Criteria. Point mutations in the *MLH1* and *MSH2* genes were screened by denaturing high performance liquid chromatography (DHPLC) followed by direct sequencing.

**Results:** Two novel nonpathogenic mutations (2 of 20 [10%]) in *MLH1*-exon 15 (intron) nt. 1707 a>g and *MLH1*-exon 13 (intron) nt. 1632 g>a were identified in brazilian CRC patients.

**Conclusion:** According to the Human Gene Mutation Database (HGMD) and the International Society of Gastrointestinal Hereditary Tumors (InSIGHT) Database, this is the first report of this mutation.

**P04.029****Characterisation of familial breast tumours using array-based comparative genomic hybridisation technology: the common heterogeneity with sporadic breast tumours**

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Familial breast cancer (~5% of breast cancer cases) maybe due to mutations at the BRCA1 and BRCA2 genes (~30% of families with breast cancer) or to mutations in other unknown genes to date in the non-BRCA1/2 or BRCAx families (~70%). To characterise the genomic differences amongst the distinct breast cancer classes using the array-based comparative genomic hybridization technique, we have collected 20 tumours associated with BRCA1 mutation, 24 tumours from mutation carriers in BRCA2, 32 BRCAx-tumours, and 19 sporadic samples.

Breast tumours associated with mutations in BRCA1 or BRCA2 had greater genomic instability than BRCAx or sporadic samples, and a trend to alter specific chromosomal regions. However, we have demonstrated a common heterogeneity between familial and sporadic breast cancer in terms of estrogen receptor (ER) status and breast cancer subtypes. First, ER-negative tumours showed higher genomic instability and a specific genomic aberration pattern compared with ER-positive tumours. Second, familial breast tumours have been profiled into molecular subtypes: basal, ERBB2, luminal A, and luminal B. BRCA1-tumours were associated with the basal phenotype, which presented the highest genomic instability, whereas BRCAx samples were related to the luminal A subtype. Luminal B tumours had the greatest number of high-level DNA amplifications.

Two regions with high-level DNA amplifications in familial breast cancer have also been studied: 8p11-p12 and 13q34. The former one was related to higher cell proliferation, whereas the latter one was characteristic of basal tumours, had 1'6Mb length, and was correlated with overexpression of candidate genes such as TFPD1.

**P04.030****BAX gene mutations and Breast carcinoma: A study of phenotype-genotype correlation**

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It has been known for a while that the proapoptotic BAX protein induces cell death by acting on mitochondria and its gene is located on 19q13.3-q13.4. On the other hand it has been demonstrated that BAX can be essential for death receptor-mediated apoptosis in cancer cells

In this study a kind of phenotype-genotype correlation research for breast carcinoma and BAX gene mutations has been done. The correlation between breast cancer stage and its prognosis has been evaluated with the mutation types of the BAX and their statistical analyses were the matter of interest through a series of clinical and genetic variables. The sample consists of 50 female patients with mean age of 40-50. who involved with Breast Cancer compared to a healthy control group of the same race and ethnicity with approximately the same mean age who also never had a history of breast cancer in their close relatives.

Our study shows some basic findings to make a Bax mutation database phenotypically interrelated with the clinical prognoses which could be a fundamental guide to clinical treatment options through a series of genetic screening.

**P04.031****Preliminary genetic investigations of BRCA genes within hereditary breast and ovarian cancer (HBOC) families in north-eastern Romania**

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BRCA1 and BRCA2 are major cancer predisposition genes, responsible for a large percentage of hereditary breast and ovarian cancer (HBOC) families. Screening for mutations in these genes is now standard practice for HBOC cases in Europe, and permits medical follow-up and genetic counselling adapted to the needs of individuals in such families. As very little information is available in Romania, we started the first characterization of hereditary breast and ovarian cancer risk in north-eastern Romania. Our study consists in HBOC families identification and recruitment, DNA collection achievement from these patients, implementation of molecular technology for mutations analysis and oncogenetic follow-up of mutations bearers.

We managed to identify and recruit so far several HBOC families with unique features such as 5 ovarian plus 1 breast cancer, or 8 breast cancer cases within the same family line. All recruited families are now under systematic DNA sequencing for BRCA screening.

We also investigated, within HBOC families and sporadic cases, the status of three known founder mutations (185delAG and 5382insC on BRCA1 and 6174delT on BRCA2), by optimization and comparison of several multiplex-PCR techniques. This part of our study showed a very important random factor within all multiplex-PCR methods. We demonstrate that although they are cheap, rapid and easy techniques, they often generate false results like primer dimmers, undesirable amplification products apparition or heteroduplexes. Therefore we always recommend combining multiplex-PCR with other pre-screening methods (SNP, heteroduplex analysis) for a good selection before DNA sequencing.

Our study was possible by financial support from Romanian Academy.

**P04.032****Implementation of a comprehensive strategy for mutational analysis of BRCA1 and BRCA2 genes in our clinical setting:****Description of the algorithm and preliminary results**

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We have recently set up an accurate and cost-effective strategy to screen for variations in the BRCA1 and BRCA2 genes. Our algorithm consists in a sequential cascade of complementary techniques using genomic DNA. First, we study copy number variations by using commercial MLPA kits (MRC-Holland). Samples showing positive MLPA results are always confirmed by a different approach. Samples with no copy number variations undergo SNplex analysis, designed specifically to detect 38 Spanish common polymorphisms and 9 recurrent mutations. Direct sequencing is applied to confirm recurrent mutations. Negative samples for the previous steps are submitted to our chosen technique for mutational search for nucleotide changes, the CSCE approach (conformation sensitive capillary electrophoresis). CSCE analysis allows detecting altered electrophoresis patterns when DNA changes are present in heterozygosity in a given fragment. The screening of the whole coding region of both genes is performed by analysing 79 different fragments. Abnormal patterns are sequenced to determine the DNA change responsible for the observed mobility shift. When a DNA change is suspected to alter the correct splicing of the genes, mRNA studies are performed. We are applying this comprehensive approach since July 2007 in our routine setting and although numbers are still too low, results are promising and comparable to those reported in the literature. We detected about 2-3% copy number alterations, 3-5% recurrent mutations and 15-18% point mutations in a selected population of high-risk patients. Several mutations affecting the splicing have been also characterized at mRNA level.

**P04.033****Rapid screening of BRCA1 and BRCA2 Spanish mutations by High Resolution Melting analysis**

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**Background:** *BRCA1* and *BRCA2* are the major genes responsible for hereditary breast (BC) and/or ovarian cancer. Since *BRCA1/2* are large genes the majority of mutation detection procedures include screening methods which are time-consuming and expensive. High-resolution melting (HRM) is a recent promising screening method that combines the simplicity, cost-effectiveness and rapid identification of genetic variants. The aim of the study is to evaluate HRM in the screening of the reported Spanish mutations.

**Methods:** We studied 40 *BRCA1* and 47 *BRCA2* DNA samples containing different Spanish mutations. We also included a reference group of 20 unknown DNAs from patients with sporadic BC. The assay was carried out on LightCycler 480 (Roche) using LightCycler® 480 HRM Master and performing 21 and 25 independent PCR for the study of *BRCA1/2* gene mutations, respectively. The melting analysis was performed with LightCycler® 480 Gene Scanning Software v.1.0. All mutations and genetic variants detected were confirmed by sequencing the PCR products.

**Results:** The HRM could discriminate the 87 *BRCA1/2* Spanish mutations analyzed from wild-type DNA. Besides, 82 out 87 mutations were clearly differentiated from each other. In the sporadic BC group we did not detect any deleterious mutations, however, we detected polymorphisms and unclassified variants in both genes.

**Conclusions:** HRM is a valuable method for rapid screening of *BRCA1/2* Spanish mutations. Besides, this procedure allows the genotyping of different mutations included and is capable of differentiating unknown genetic variants present in the PCR product.

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**P04.034****Novel deleterious *BRCA1/2* mutations found in the population of Eastern Spain**

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From the identification of *BRCA1* and *BRCA2* as major genes responsible of hereditary breast and/or ovarian cancer more than 1,800 pathogenic mutations have been reported in the Breast Cancer Information Core, many of them related with ethnic and/or geographic conditions (Neuhausen SL, 1999). The objective of the study is to describe the twenty novel deleterious mutations identified in the Valencian Community.

In the three first years of performance of the Program of Genetic Counselling in Cancer of the Valencian Community we studied the *BRCA1* and *BRCA2* mutations in 596 unrelated breast/ovarian cancer patients. The method followed consisted of multiplex PCR amplification of all exons of the genes, mutation screening by the study of homoduplex/heteroduplex and mutation identification by sequencing of the PCR products.

We found 52 different deleterious mutations (21 in *BRCA1* and 31 in *BRCA2*), among the 112 positive index patients. In *BRCA1* gene we found 12 frameshift, 3 nonsense, 3 missense and 3 splicing mutations. In *BRCA2* we found 24 frameshift, 5 nonsense and 2 splicing. Twenty of these mutations have not been reported previously, 5 in *BRCA1* (c.1689delG, c.3700delC, p.Y1429X, c.4424\_4425delCT and c.5430\_5452del23) and 15 in *BRCA2* (c.489\_490delCT, c.1835insT, c.1874\_1877delAGGA, c.2929delC, c.5025delT, c.5344\_5347delAATA, c.5946\_5947dupCT, c.6118delA, c.6722delT, c.7400insA, p.Y2621X, c.8270\_8271delCA, c.8860+2T>G, c.9218delA and p.Q3156X).

From our data two main conclusions could be drawn: (a) the remarkable proportion of novel mutations (20/52; 38.4%) and (b) that the majority of these mutations (15/20; 75%) were found in *BRCA2* gene.

**P04.035*****BRCA1* and *BRCA2* variability in breast/ovarian cancer families from the Basque Country**

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*BRCA1* and *BRCA2* are tumor suppressor genes that are related with inherited susceptibility to breast/ovarian cancer. We are analyzing the polymorphic variants of *BRCA1* and *BRCA2* genes in families with clinical criteria of hereditary breast/ovarian cancer. After DNA isolation, the mutational analysis were performed by DNA sequencing of all *BRCA1* and *BRCA2* exons and flanking regions. Twentythree different types of *BRCA2* and twenty *BRCA1* polymorphic mutations were identified in twenty breast/ovarian cancer families. The patients selection was made after a genetic counseling procedure, according the diagnostic criteria recommended by de Spanish Society of Medical Oncology (SEOM) and also using the BRCAPRO informatic model.

**P04.036****Novel and common *BRCA1* mutations in familial breast/ovarian cancer patients from Lithuania**

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**Background:** Mutations in the *BRCA1* gene are found in many families with multiple cases of breast and ovarian cancer. Twenty seven unrelated female patients diagnosed with breast cancer and/or ovarian cancer have attended cancer genetics clinic during period of 2006-2007 year.

**Methods:** *BRCA1* gene testing was initiated with direct sequencing for common founder mutations: 4153delA in exon 11, 5382insC in exon 20 and enzyme restriction digestion for 300T/G in exon 5. After the exclusion of common mutations, multiplex ligation-dependent probe amplification (MLPA) and prescreening by denaturing gradient gel electrophoresis (DGGE) with confirmation by direct sequencing were performed.

**Results:** Overall, pathogenic *BRCA1* gene mutations were prevalent in 8 (30%) unrelated proband patients: 3 (15%) with breast cancer (two 5382insC, one 4153delA), 2 (50%) with ovarian cancer (5382insC and 4153delA each) and 3 (100%) with breast and ovarian cancer (5382insC, 4153delA and novel previously not reported deletion 4635delG in exon 15). No 300T/G mutation and *BRCA1* rearrangements were detected.

For patients diagnosed with breast cancer ≤35 year, *BRCA1* mutation detection rate was 27% (3/11) and mutations were absent in breast cancer patients group diagnosed after 36 years (0/6).

**Conclusions:** Testing for two common *BRCA1* mutations should be considered in female cancer cases\* when: (a) breast cancer diagnosed before the age of 36 year; (b) medullary breast carcinoma; (c) breast and ovarian cancer; (d) nonmucinous epithelial ovarian tumor regardless the age (\*or first degree relative/second through male). Full *BRCA1* gene analysis is warranted in patients with strong family history of breast cancer.

**P04.037****Occurrence of *BRCA1* cryptic splice site mutation: IVS5-12A>G in hereditary breast cancer families in the Czech republic**

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High Resolution Melting (HRM) analysis revealed presence of *BRCA1* cryptic splice site mutation IVS5-12A>G in high-risk breast/ovarian cancer patients. This change was described to form a cryptic splice site resulting in the addition of 11 nucleotides at the intron 5/exon 6 boundary. The *BRCA1* mutation IVS5-12A>G was not detectable by the previously used heteroduplex analysis (using MDE gel) at the MMCI. Therefore, the retrospective study of 480 high-risk familial breast/ovarian cancer patients without previously identified *BRCA1/2* mutation were analysed for exon 6 by HRM using LightScanner instrument. The *BRCA1* mutation IVS5-12A>G was found in 11 high-risk probands. Adjoining these new findings to our previous results, the IVS5-12A>G mutation in *BRCA1* gene represents 5,3% of all *BRCA1*

mutations detected in hereditary breast cancer families. All together, the mutation detection rate in BRCA1 or BRCA2 genes reached 39,8% in hereditary breast cancer families (309 families carrying BRCA mutation out of 778 analysed families with at least 2 diagnosed breast and/or ovarian cancer cases before the age of 50).

Any report about presence of BRCA1 mutation IVS5-12A>G in Slavic population is missing in the BIC database. Therefore other methods were tested for their capability to detect this mutation. We found that HRM as well as DHPLC can perfectly detect the IVS5-12A>G mutation, however detection with SSCP highly depends on running condition. By using SSCP or HA the BRCA1 mutation IVS5-12A>G might be easily overseen. *Supported by the Ministry of Health of the Czech Republic: Grant MZOMOU2005*

#### P04.038

##### Expression of BRCA1 and TP 53 genes in different types of tumor

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The TP53 is one of the main suppressors in many types of tumor ailments. BRCA1 is another tumor suppressor gene involved in DNA repair and it is best known by its role in the development and proliferation of various types of breast and ovarian cancers. Previous studies indicate that mutations in these genes are related to pathogenesis and development of the disease.

Aim of our study was to determine if there is any relation between expressions of these two genes and also to determine level of expression of BRCA1 gene in colon cancer. Expression level of TP53 in different cancers was compared to the expression of BRCA1 and beta -actin that was used as a control gene. This study included samples from 76 subjects with breast, ovarian and colon cancer. Genetic material was extracted from bioptic specimens retrieved from Department of Pathology from University Clinical Center Sarajevo. Expression of the observed genes was measured by real time PCR on Applied Biosystems 7300 device using SYBR green method.

#### P04.039

##### Contralateral prophylactic mastectomy in BRCA1/2 mutation carriers with breast cancer

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The occurrence of bilateral breast cancer in BRCA1/2 mutation carriers varies subject to factors such as age at diagnosis of 1<sup>st</sup> tumor and family history for breast-ovarian cancer. BRCA1/2 mutation carriers are counseled to undergo prophylactic mastectomy (PM) and oophorectomy (PO). The incidence of contralateral prophylactic mastectomy was evaluated in BRCA1/2 mutation carriers diagnosed with unilateral breast cancer from one oncogenetic clinic in Northern Israel.

Socio-demographic and clinical profiles of 59 eligible BRCA1/2 mutation carriers were collected. Women who opted for contralateral PM were compared to those who choose medical surveillance.

Of the 59 women, three (5.1%) underwent contralateral PM and 33 (55.9%) underwent PO and five (8.5%) had both contralateral PM and PO. The three women who underwent contralateral PM were younger than 40 years of age at diagnosis. Mean age at diagnosis was not statistically different between those who opted for PM and those who preferred surveillance. However, women who chose contralateral PM were significantly younger than those who preferred follow-up ( $48.12 \pm 5.27$  and  $59.81 \pm 9.96$ , respectively,  $p=0.001$ ). The only socio-demographic variable found to be associated with PM was graduate level of education; all eight women had academic education.

These findings suggest that most breast cancer patients diagnosed with BRCA1/2 mutation declined PM. It is yet to be clarified whether preferences of risk reduction management in Israel, are subject to woman's prevailing values or medical recommendations.

#### P04.040

##### Chromosomal damage after *in vitro* treatment with radiation or mitomycin c in lymphocytes of BRCA1/BRCA2 mutation carriers

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**INTRODUCTION AND OBJECTIVES:** Mutations in *BRCA1* and *BRCA2* account for a fraction of inherited susceptibility to breast (BC) and ovarian cancer (OC). The *BRCA1/BRCA2* gene products help to maintain genomic integrity after DNA damage. To investigate this capacity in *BRCA1/2* heterozygous cells, we have evaluated their *in vitro* sensitivity to chromosomal damage induced by two mutagenic agents: ionising radiation and mitomycin C (MMC).

**STUDY SUBJECTS AND METHODS:** Lymphocyte cultures were established from 20 *BRCA1* mutation carriers (12 with cancer and 8 without), 21 *BRCA2* mutation carriers (11 with cancer and 10 without) and 11 non-carrier controls. The chromosomal damage (chromosome breakage and chromosome loss) induced *in vitro* was assessed quantifying bi-nucleated cells with micronuclei (MN). MN can be used as a measure of DNA repair capacity. Six cultures were set up for each subject (2 treated with 2Gy gamma irradiation from Co-60, 2 treated with 0,05 µg/ml of MMC and 2 without treatment).

**RESULTS:** The *BRCA1/BRCA2* heterozygous lymphocytes did not show a higher level of radiation-induced MN compared to the non-carrier controls lymphocytes. In contrast, the *BRCA2* lymphocytes, from both women with and without cancer, presented higher levels of MN after exposure to MMC than the lymphocytes of *BRCA1* carriers and women without mutation.

**CONCLUSION:** The haploinsufficiency of *BRCA1/BRCA2* in peripheral blood lymphocytes does not affect the repair of radioinduced DNA lesions. But the absent of one *BRCA2* functional allele is associated to a higher level of chromosomal damage induced by MMC probably due to an impaired DNA repair capacity.

#### P04.041

##### The prevalence of three mutations in BRCA genes in Romanian women with familial breast cancer

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**Background.** BRCA1 and 2 mutations confer a substantial lifetime risk to breast and ovarian cancer. The spectrum of mutation in these genes has been not previously investigated in our country.

**Aim.** To evaluate the prevalence of BRCA1 and BRCA2 mutations in women with demonstrated history of breast cancers.

**Material and methods.** We started this study in 2007 and selected 30 Caucasian patients with breast cancer which was confirmed by clinical and paraclinical approach. All patients have at least one relative with early breast cancer onset. Signed informed consent for all participants was obtained before inclusion in study. We used blood samples to test the presence of BRCA1 185 AG, BRCA1 5382 insC, BRCA2 6174 delT mutations using commercial kit and classical PCR based methods. In addition we screened by indirect methods a region of BRCA1 (1000 bp) for other mutations.

**Results.** We identified the heterozygous BRCA1 5382 insC mutation in three patients, confirmed by both methods. The other two mutations have been not detected. Thus, we consider that both methods have similar efficiency in detection of these mutations. We also found no mutations in investigated regions using indirect methods. Conclusion. Our preliminary results showed that BRCA1 5382 insC could be the most common mutation in Romanian women with familial breast cancer.

**P04.042****BRCA1/2 mutations: screening strategy for the analysis of Portuguese high-risk breast/ovarian cancer families**

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**Background:** Five to ten percent of breast cancers are hereditary and 50-80% due to BRCA1/2 mutations. Because screening of BRCA1/2 mutations is complex and indeterminate results are frequent, only high-risk patients should be counselled for this diagnosis. The possibility of an informative result also depends on diagnostic methodologies performed. Purpose: elaboration of an algorithm for BRCA1/2 mutation screening in Portuguese high-risk breast cancer families. Methods: Integration of clinical and previous molecular data showing that c.156\_157insAlu in the BRCA2 gene is a Portuguese founder mutation , BRCA1 immunohistochemistry helps in selection for screening as well as the prevalence of multiplex ligation dependent probe amplification (MLPA) detected rearrangements . Results: We suggest the following algorithm: 1-All samples are first screened for the founder mutation (6-8% positives); 2- if negative in 1 recurrent rearrangements (g.Ex13ins6kb and g.Ex11\_15del) are screened by PCR; 3- negatives in 1 and 2 are screened by conformation sensitive gel electrophoresis. The first gene to analyze depends on tumor phenotype: BRCA1 first if BRCA1 immunohistochemistry is negative and/or is a triple negative tumor. BRCA2 first if a BRCA1 phenotype is excluded and/or history of male breast cancer or BRCA2 associated tumours (gastric, M. mieloma, pancreas); 4- if still negative MLPA is performed.

**Conclusion:** This algorithm might fit into a complete screening program and be superior to current practice in terms of providing more informative results.

**P04.043****Correlation between BRCA1 mutations and BRCA1 expression level in breast cancer specimens**

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Tumor suppressor BRCA1 gene is associated with increased risk for developing breast and ovarian cancer. Approximately 80% of hereditary breast cancer cases have mutations in BRCA1 gene. This gene is expressed in several organs, including breast and ovary. BRCA1 encodes a 220 kDa protein which consist of 1863 amino acids. Expression of BRCA1 gene is decreased in the most early - detected sporadic breast cancer cases, and it decreases continuously with degree of malignancy.

The goal of this experiment was to investigate whether expression of BRCA1 is affected by mutations on BRCA1 gene. Tumor biptic specimens from patients fulfilling criteria to belong to high to medium risk group for breast cancer development have been consecutively collected at Sarajevo Clinical Center during the period of two years. DNA was isolated from tumor tissue samples using standard salting out procedure. Mutations in BRCA1 gene were identified by MLPA reaction. For expression level measurement, RNA from tumor specimens was isolated using guanidine isothiocyanate method, followed by reverse transcription. Measurement of expression levels of BRCA1 gene was performed in 7300 Real Time PCR system (Applied Biosystems) with SYBR green method. After collecting data, correlation calculations were performed. Results from MLPA analysis were correlated with expression levels of BRCA1 gene. Results are displayed in graphic figures.

**P04.044****The 3-step PCR methodology is the correct option to screen the c.156\_157insAlu BRCA2 Portuguese founder mutation**

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**Introduction:** The insertion of an Alu fragment in position c.156\_157 of BRCA2, causing exon 3 skipping, was confirmed to be a Portuguese founder mutation and the most frequent BRCA2 rearrangement described. We also optimized a methodology for the screening of this mutation. **Patients and methods:** All new high-risk patients counselled for BRCA1/2 screening in our Centre are pre-screened for this mutation with a 3-step PCR method (Patent pending): 1) exon 3 PCR and sequencing, 2) nested PCR with primers 3AluF/3AluR and 3) RT-PCR and cDNA sequencing. Other Centres, in Portugal and abroad, are implementing this PCR-based method to screen for this mutation. Additional family inquiries are made due to the founder effect of this mutation. **Results:** Twenty-nine apparently nonrelated Portuguese c.156\_157insAlu-positive families were diagnosed in our Centre and one BC/OC family of Portuguese ancestry was also diagnosed in Caen, France, using our method. Also, a first degree relative of one of our new probands had been studied in USA and diagnosed with an unknown variant (Q2731E). Exhaustive family inquiries allowed us to connect 3 of these new and apparently non-related families with families already identified. **Conclusion:** The correct option to screen the c.156\_157insAlu BRCA2 Portuguese founder mutation is the 3-step PCR method we described. Our data suggests that other methodologies may miss this mutation, preventing these families from obtaining an informative result.

**P04.045****Prevalence of large rearrangements in Portuguese hereditary breast/ovarian cancer families**

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**Background:** Hereditary breast/ ovarian (HBO) cancer is mainly related with BRCA1/2 mutations. Given the increasing number of rearrangements reported and the observation that the most frequent BRCA mutation in our country is a BRCA2 rearrangement (c.156\_157insAlu), present in about 50% of our BRCA1/2 positive families, the contribution of other large genomic rearrangements to the Portuguese BRCA mutation spectrum must be analysed. Aim: to evaluate the frequency of large rearrangements in the BRCA1/2 genes in Portuguese HBO families. **Patients and methods:** Ninety high-risk families, previously negative for BRCA1/2 mutations by conformation sensitive gel electrophoresis, were screened for the g.Ex13ins6Kb BRCA1 by PCR using specific primers and multiplex ligation-dependent probe amplification (MLPA). **Results:** Two additional rearrangements, both in the BRCA1 gene, were observed: g.Ex13ins6Kb (1 family) and g.Ex11\_15del (1 family), accounting for 22% (2/9) of the BRCA1 mutation spectrum in our families. With the MLPA BRCA2 kit, 1 patient was found to be positive for the 1100delC mutation in CHEK2 and 3 patients had copy number changes in this gene. No large BRCA2 rearrangements were observed. **Conclusion:** Besides the screening of the founder BRCA2 rearrangement in our population, index high-risk cases negative for BRCA1/2 point mutations should be analysed for other possible genomic BRCA rearrangements. Although infrequently, CHEK2\*1100delC is present as a low penetrance allele in our population. Additional studies are necessary to clarify the relevance of copy number changes in CHEK2 gene.

**P04.046****Zoom-in Array-CGH in BRCA1, BRCA2, MLH1, MSH2 and APC genes : detection and characterization of five new germline large rearrangements**

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Genetic predisposition to breast, ovarian, colorectal cancers results mainly from alterations in BRCA1, BRCA2, MLH1, MSH2 and APC genes. Except for BRCA2, rearrangements represent 10 to 15% of

deleterious mutations. Many routine detection techniques are available, but none gives a panoramic view of the gene and characterizes breaking points.

We have developed a zoom-in array-CGH for detecting and characterizing rearrangements based on Agilent® high-resolution oligonucleotide array-CGH technology. The Centre René Huguenin designed three specific arrays for the *BRCA1* (1679 oligonucléotides) & *BRCA2* (1389), *MLH1* (1031) & *MSH2* (789) and *APC* (1573) genes.

Prior to use this approach in routine, we analyzed 18 DNA samples with a large deletion or duplication detected with QMPSF, MLPA or qPCR : 10 *BRCA1/BRCA2*, and 3 *MLH1/MSH2* from Centre René Huguenin and 4 *APC* from Institut Paoli Calmettes.

All the large rearrangements from single small exon to the whole gene were detected (size estimated to within 1-2 kb). Five never-reported events were characterized (table). Except for the whole *APC* gene deletion, the event was characterized thanks to a simple PCR.

Zoom-in Array-CGH described here gives the opportunity to rapidly screen a group of genes involved in breast and colorectal cancer. Despite its cost, this method can assist with the development of simple PCR-based genetic test. Additional studies are needed to define its position in routine testing.

Five new germline large rearrangements characterized with zoom-in array-CGH and sequencing

Genes	Events	Size
<i>BRCA1</i>	deletion exon 3	11 452 bp
<i>BRCA2</i>	duplication exons 17-20	14 756 bp
<i>APC</i>	deletion exons 12-14	6 360bp with insertion 351bp
<i>MLH1</i>	duplication exon 4	1 664 bp
<i>MSH2</i>	deletion exons 8-10	26 349 bp

#### P04.047

##### Estrogen receptor- $\alpha$ (*ESR1*) gene polymorphisms in Iranian women with breast cancer: a case control study

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Receptor-mediated estrogen activation participates in the development and progression of breast cancer. Evidence suggests that alterations in estrogen signaling pathways, including *ESR1* occur during breast cancer development. *ESR1* polymorphism has been found to be associated with breast cancer in Caucasians. Epidemiologic studies have revealed that age-incidence patterns of breast cancer in Middle East differ from those in Caucasians. Genomic data for *ESR1* in either population is therefore of value in the clinical setting for that ethnic group and we have investigated whether polymorphisms in the *ESR1* are associated with breast cancer risk.

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 individuals). PCR single-strand conformation polymorphism technology and <sup>33</sup>P-cycle sequencing was performed.

The sets of silent single nucleotide polymorphisms were found, as reported previously in other studies, but at significantly different frequencies. Among these SNPs, the frequency of allele 1 in codon P325P (C1337G) was higher in breast cancer patients (10.3%) than in control individuals (8.8%;  $P=0.538$ ), although the difference was not statistically significant. The allele 1 in codon H267L (A1162T) exhibited an association with the occurrence of family history of cancer among breast cancer (0.8%;  $P=0.004$ ).

Our data suggest that *ESR1* polymorphisms are correlated with various aspects of breast cancer in Iranian *ESR1* genotype.

#### P04.048

##### EMQN Best Practice Guidelines for Molecular Analysis in Hereditary Breast/Ovarian Cancer

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Breast cancer is the most frequent form of cancer in women. Genetic predisposition underlies 5-10% of all breast cancer and up to 40% of cases in patients under the age of 35 years. The discovery of *BRCA1* and *BRCA2* in 1994 and 1995, respectively, was anticipated to greatly increase our knowledge of hereditary breast cancer. This was immediately followed by an increasing demand for genetic testing from health care to predict future cancer risks. Since then, breast cancer genetics has become a major part of the workload of clinical genetics clinics. The identification of the *BRCA1/2* mutations and the communication of the genetic information are essential for women at high risk for breast cancer. As a result, guidelines for genetic counselling and laboratory quality assessment in cancer genetics have been developed at local and national levels both in Europe and in the USA. The European Molecular Genetics Quality Network (EMQN) drafted best practice guidelines on breast/ovarian cancer genetic testing as a first attempt towards European harmonisation in 1999. In 2007, OECD has issued general recommendations for molecular genetic testing. The best practice guidelines presented here are the results of discussions among European breast cancer geneticists at a workshop in Würzburg, Germany, October 24-25, 2007 with the aim of updating the 1999 EMQN guidelines.

#### P04.049

##### *HFE* and *TFR* polymorphisms in the risk of breast cancer in São Miguel population (Azores, Portugal)

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Iron overload has been noticed as a feature of breast cancer (BC). *HFE* mutations, which lead to iron uptake increase, have been evaluated as risk factor. Some studies concluded that BC risk increase is also dependent on *HFE*-interacting genes, such as the transferrin receptor (*TFR*) and the combination between *HFE-TFR* genotypes.

To assess if *HFE* mutations, *TFR*-S142G polymorphism and *HFE-TFR* genotypes are related to BC risk, we compared C282Y, H63D and S142G frequencies in 86 BC women and in 183 gender/age matched healthy controls. Samples were obtained after written informed consent.

The C282Y allele frequency in the BC group was 4.07%, higher than in control group, 3.28%; while H63D mutation showed a similar frequency in BC, 21.51%, and controls, 21.04%. Although both groups were stratified according to menopausal status, odds ratio (OR) analysis for cancer risk associated with *HFE* mutations was not statistically significant. Regarding S142G polymorphism, the frequency of S142S, S142G and G142G genotypes were equivalent in both groups. In order to extend the search for a supposed BC susceptibility for *HFE-TFR* genotypes, we analysed BC and controls according to compound genotypes. Again, OR for all *HFE-TFR* genotype combinations revealed no increased risk for BC.

In conclusion, the results suggest that *HFE* mutations are not associated with an increased risk for BC. *TFR* polymorphism was not an independent risk factor and did not modify the disease risk. Furthermore, variants of the *HFE-TFR* have, apparently, no direct effect on the incidence of breast cancer in the Azorean female population.

**P04.050****Chromosomal aberrations in Breast cancer patients in Tamilnadu Region**

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Breast cancer is the most common cancer in women and accounts for between 18-25% of all female malignancies world-wide. In India, the incidence of breast cancer is increasing, with an estimated 80,000 new cases diagnosed annually. The frequency of chromosome instability in peripheral blood lymphocytes is relevant biomarker for cancer risk in humans. So, the present investigation aimed to find out the major chromosomal alteration in breast cancer patients in Tamilnadu region by using the conventional karyotyping method. 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. The work was carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. After signing a consent form, both cases (experimental and controls) provided a blood sample (5 ml) to establish the 72hrs cell cultures.

In the present study deletion and translocation were frequently observed in chromosome 1, 11 and 17. (46, XX, del (1p); 46, XX, del (11q); 46, XX, del (17q). 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>)).

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.

**P04.051****Characterization of novel large genomic rearrangements in the BRCA1/2 genes of Spanish families with breast/ovarian cancer**

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**Background:** Mutations in *BRCA1/2* genes affecting a small number of nucleotides are the responsible for the majority (90%) of hereditary breast and/or ovarian cancers (HBOC). However, there is an increasing evidence of the contribution of large genomic rearrangements (LGRs) of *BRCA1/2* genes to HBOC.

The purpose of this study is to characterize the novel LGRs identified in *BRCA1/2* genes and determine its prevalence in families studied in Program of Genetic Counselling on Cancer of the Valencian Community (Spain).

**Method:** We used the multiplex ligation-dependent probe amplification (MLPA) to screen for LGRs in 255 unrelated index patients with familial breast and/or ovarian cancer negative for *BRCA1/2* mutations. Characterization of the LGRs was carried out by performing long-range PCR followed by sequencing.

**Results:** Nine different LGRs were identified in once index patients out of the 225 (4.3%), seven in *BRCA1* and two in *BRCA2*. Five *BRCA1* LGRs have been already reported (1A/1B and 2 deletion; 5 to 7 deletion; 8 to 13 deletion, exon 20 deletion and amplification of exon 20) and also four novel LGRs were found. Characterization of the novel mutations revealed the presence of deletions that encompass exons 3 to 5 of *BRCA1*, partial deletion of exon 21 of *BRCA1*, deletion of exons 22 to 24 of *BRCA2* and deletion of exon 2 of *BRCA2* gene.

**Conclusions:** These findings contribute to broaden the spectrum of LGRs described in the *BRCA1/2* genes. Additionally, our data emphasize the relevance of LGRs in the mutational study of *BRCA1/2* genes of high-risk families.

**P04.052****UNC5 genes and breast cancer: a mutation screening and LOH analysis**

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Dependence Receptors (DR) display the common feature of inducing two completely opposite intracellular signals: in the presence of the ligand, they transduce a positive survival signal, whilst in its absence, they induce apoptosis. The three mammalian receptors named UNC5A, UNC5B, and UNC5C in human, belong to the family of the netrin-1 receptors, act as mediators of netrin-1 chemorepulsive effect during neural development, but also work as dependence receptors. Expression of human genes is down-regulated in multiple cancers including colorectal and breast cancers. In colorectal tumors, this down-regulation is associated with loss of heterozygosity (LOH). In order to verify the possible LOH in breast cancer, a panel of 57 breast tumors were analysed for these genes using intragenic microsatellite markers, but no LOH was detected. Since mutations impairing mRNA or protein levels would not have been detected by LOH analysis only, a mutation screening of PCR amplified exons is currently ongoing. In UNC5B a new variant was identified in exon 10, T1909C, which results in a silent substitution in the protein but by *in silico* analysis affects an exon splicing enhancer (ESE), which could modify mRNA processing. The change is now tested in a panel of 100 controls. Depending on the results obtained, the variant will be further investigated using a splicing assay with a synthetic minigene. The analysis is still ongoing and final results will be presented.

**P04.053****Misbehaviour of XIST RNA in breast cancer cells**

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Recently, a strong open debate has been dragging on about the role of the *BRCA1* in the XCI (X chromosome inactivation) process and about the meaning of the "Disappearing Barr Body" observed in breast and ovarian cancer. A definitive answer about the hypothetical function of *BRCA1* in the correct localization of XIST RNA on the inactive X chromosome (Xi) hasn't been found yet, while it's known that in breast cancer, especially with basal like phenotype, can be frequently observed X chromosome anomalies, mainly Xi loss associated to replication of the active X chromosome (Xa).

We investigated some aspects to further sound out the supposed link between *BRCA1* and XIST expression/localization. In addition, we tested the possibility of XIST RNA to associate with a different target than Xi, in presence of anomalous XCI status, a common feature of breast carcinomas. The study was performed on tumor cell lines and breast carcinomas by a combined genetics and epigenetics approach.

We drown the following conclusions: i) high levels of XIST RNA were observed as a marker of *BRCA1*-associate BLC; ii) *BRCA1* was involved in regulation of XIST expression on the Xa, but we confirmed the absence of a link between *BRCA1* and XIST nuclear distribution; finally, we surprisingly surveyed in breast cancer cells the ability of XIST RNA to decorate an active X chromosome, demonstrating that the presence of focal XIST staining in the nucleus does not prove the existence of an inactive X chromosome.

**P04.054****Genetic peculiarities of families with inherited bilateral breast cancer**

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Bilateral breast cancer (BiBC) is considered as phenotypic sign of inherited breast cancer risk. However it is unknown if there are any peculiarities of familial breast cancer (BC) with cases of BiBC. We investigated BRCA1/2 mutations among 38 patients with BiBC who had one or more relatives with BC or ovarian cancer (OC). The BRCA1/2 mutations were found among 19 patients (50%). The BRCA1 gene mutations were predominant (16 of 19, 84%) with prevailing 5382insC (9 of 16, 56%). Two BRCA1 gene mutations - 3411delCT and 3747insA and three BRCA2 mutations - P3039P, 9498delC, 886delGT were revealed in Russia for the first time. Among 10 BC/OC families there were 6 BRCA1 mutations that fully correspond to 60% defined earlier for the same families without BiBC. Among BiBC families with BC only 36% had BRCA1 mutations. For comparison, among 92 BC families unselected on BiBC there were 21% mutations. Another words, BRCA1 mutations in BiBC families more frequently predispose to BC only than in families without BiBC. Beside this, there were 10 cases of repeated BiBC in 8 families and all of them were BC only families. It may be proposed that in some families with BiBC there is genetic influence that modify risk of cancer localization. In part supported by grant RFBR N 07-04-01602.

#### P04.055

#### High frequency of BRCA1 gene mutations among patients with pregnancy associated breast cancer

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A relationship between a pregnancy and breast cancer risk is a subject of studying at last time. The reasons for breast cancer onset during a pregnancy are not clear at present time. A role of BRCA1/2 mutations in pregnancy associated breast cancer (PABC) onset is not well established. We studied the most frequent in Russia BRCA1 mutations 5382insC, C61G and 185delAG in a sample of 74 patients with PABC and in a control sample of 75 breast cancer patients. A control sample was adjusted on age of diagnosis among PABC patients (an average age 33 year). There were 12 of 74 PABC patients with BRCA1 mutations (16%). The patients with 5382insC mutation were predominant (11 of 12). For all but one of 12 PABC patients breast cancer was diagnosed during second or further pregnancy. Among patients of control sample there were 6 with mutations (8%), all 5382insC. The high BRCA1 mutation frequency among PABC patients could be connected with some peculiarities of this patient group. We found that the age of diagnosis for BRCA1-associated breast cancer among PABC patients was in interval 26-35 years while among patients of control group - 33-46 years. Thus the high frequency of BRCA1 mutations and early breast cancer onset among PABC patients is suggestive on undetermined now genetics or phenotypic peculiarities that may increase breast cancer risk during a pregnancy.

#### P04.056

#### CLSPN gene in hereditary susceptibility to breast cancer

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Approximately 5-10 % of breast cancer is thought to be due to an inherited disease predisposition. Mutations in currently known cancer susceptibility genes explain only 20-30% of the familial cases, which suggests the contribution of additional genes. Claspin, encoded by the CLSPN gene, is involved in monitoring of replication and sensing of DNA damage, during which it cooperates with CHK1, BRCA1 and ATR. As many previously identified susceptibility factors act in similar functional pathways as claspin, CLSPN is a plausible candidate gene for heritable breast cancer susceptibility. Here we have screened the entire coding region of the CLSPN gene for mutations in affected index cases from 125 Finnish families with breast and/or ovarian cancer using conformation sensitive gel electrophoresis (CSGE) and direct sequencing. Several sequence changes were observed, but none of them appeared to significantly associate with breast cancer susceptibility. To our knowledge, this is the first study reporting the mutation screening of the CLSPN gene in familial breast cancer cases.

#### P04.057

#### BRCA1-c.5272-1G>A and BRCA2-c.5374\_5377delTATG are Founder Mutations in the East of Castilla-León (Spain)

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The incidence of germline mutations in BRCA1 and BRCA2 genes in high-risk families for hereditary breast and/or ovarian cancer varies among different populations; presenting a wide spectrum of unique mutations, whereas in other groups specific mutations show a high frequency due to a founder effect. The mutations c.5272-1G>A in BRCA1 and c.5374\_5377delTATG in BRCA2 are the most prevalent in our region; they have been identified seven and nine times, respectively, in our population suggesting the existence of founder effects in our region.

Six highly polymorphic markers spanning BRCA1 (D17S800, D17S1185, D17S855, D17S1323, D17S1325 and D17S579) and four in BRCA2 (D13S171, D13S260, D13S1695 and D13S1698) were genotyped in 16 index cases and additional family members. The estimation of founder mutation age in generations was calculated using the equation  $G = \log \delta / \log (1-\theta)$ <sup>1</sup>.

All carriers of c.5272-1G>A shared the same alleles at the six markers of BRCA1. We could not estimate the age of the mutation because the lack of recombinant markers that are required for this purpose. A conserved haplotype was observed in c.5374\_5377delTATG positive families between the two external markers (approximately 1 Mb interval). The mutation occurred approximately 36 generations (900 years assuming 25 years per generation). The corresponding haplotype of each mutation were absent in non-carriers and 75 healthy controls supporting the hypothesis of a common ancestry. Conclusively, mutations c.5272-1G>A for BRCA1 and c.5374\_5377delTATG for BRCA2 are founder in our region.

<sup>1</sup> Risch N. et al, Nat Genet 9:152-159, 1995.

#### P04.058

#### Tissue microarray analysis of 1q21.3 and 1q23.3 copy number changes in ovarian tumors with different clinicopathological parameters

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Many studies reported that aberrations like amplifications, deletions and translocations of 1q21-q23 have been found in ovarian tumors. These findings increase the scientific interest in analyzing this region by means of specific gene probes. The object of our study was to investigate the frequency of copy number changes of two specific BAC clones in 1q21.3 and 1q23.3 in large number ovarian tumors from different malignancy, histology, stage and grade, arranged in tissue microarrays, in order to analyze their correlation with tumor phenotype. Copy number changes of 1q21.3 were established in 9.64% of malignant, in 8.33% of LMP and in 13.13% of benign ovarian tumors. Copy number changes of 1q23.3 were found in 17.78% of malignant, in 16.67% of LMP and in 12.64% of benign ovarian tumors. We have found significantly more frequent gain of 1q23.3 in non-epithelial tumors (50%) compared to epithelial ones (14.73%) ( $p < 0.03$ ). According to our results the gain of 1q21.3 prevailed in non-serous (especially mucinous) malignant and LMP ovarian tumors in comparison to serous tumors. In non-serous tumors both gains were associated with higher grade. The gain of 1q23.3 was 2.5 times more frequent than the gain of 1q21.3 in ovarian cancers.

#### P04.059

#### Complex BRCA1 and BRCA2 SNP combinations are associated with breast and ovarian cancer risk modification

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There are growing evidence that a number of gene variant combinations have important value for cancer risk. We have studied a significance of all combinations of BRCA1 E1038G and BRCA2 N372H,

203G>A among patients with sporadic breast and ovarian cancer, patients with the same types of cancer with BRCA1 mutations and a control sample. It was shown that genotype E1038E-N372N-203AA was associated with ovarian cancer risk for both sporadic and BRCA1-associated ovarian cancer (OR=6,8; P=0,04). At the same time, genotypes E1038E-N372H-203GA and G1038G-N372N-203GA were associated with decreased ovarian cancer risk (OR=0,2; 0,1; P=0,04; 0,02, respectively). In the sample of sporadic breast cancer genotype E1038E-203GA was associated with decreased risk independently on N372H genotypes (OR=0,1; P=0,0001) and G1038G-N372N - independently on 203G>A (OR=0,2; P=0,01). The sporadic breast cancer risk was increased under E1038G-N372H (OR=2,1; P=0,04). There was no difference of any genotype frequencies and their combinations between BRCA1 mutation carriers with breast cancer and control sample. This may means that no risk modifications are required for breast cancer localization under BRCA1 mutations. Thus the genotype combination increasing cancer risk is different for ovarian and sporadic breast cancer and those decreasing cancer risk are the same but degenerated on N372H under breast cancer. The results demonstrate that different genotype combination on the same SNPs may have influence on modificanting cancer risk of definite localization. The risk genotypes on several SNPs may include both heterozygotes and homozygotes as on rare so on frequent alleles. It is necessary take into account under polygene influence analysis.

#### P04.060

##### Molecular cytogenetic analysis of malignant ovarian tumours

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Ovarian cancer represents almost 30 % of the malignancies of the female genital tract with the highest mortality of all of the gynaecological cancers. Unfortunately, majority of patients is diagnosed at advance stage of the disease. The genetic changes involved in pathogenesis of ovarian cancer are still not completely understood. Currently there are no specific prognostic markers for prediction of the disease course, for earlier diagnostics or for individual therapeutic strategies. One of possible biological markers of great importance are chromosome changes. Chromosome aberrations in ovarian tumour cells are highly complex with hypodiploid or polyploid constitution. The aim of the study is to determine significant chromosome changes as reliable predictive markers.

We examined 30 ovarian cancer samples by comparative genomic hybridization. Chromosome imbalances were detected in 90 % of tumour samples. The most frequent recurrent changes were gains of 1q, 3q, 8q and 20q and losses of 4p, 4q, 18p, 18q, 19q and 22q. The results of molecular cytogenetic analysis were correlated with histological/morphological and clinical findings. Summarized data showed that significance of chromosome changes in our patients is relatively low. Despite of these results particular chromosome regions were assumed to be involved in ovarian cancerogenesis. These regions are worthy of further investigations considering the presence of candidate genes.

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#### P04.061

##### Aberrant promoter methylation of GPR150, ITGA8 and HOXD11 in ovarian cancers induced by PRTFDC1 silencing

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It is known that methylated CpG island (CGIs) in promoter region could be seen in tumor-suppressor genes and disease markers. In this study, we performed a genome-wide screening for altered methylated DNA fragments with methylation-sensitive-representational difference analysis (MS-RDA) to show aberrant promoter methylation of CpG island in human ovarian cancers. We have obtained 185 DNA fragments specifically methylated in an ovarian cancer cell line (ES-2). We used a normal human ovarian epithelial cell line, HOSE6-3 as a control. In this control cell line, 33 DNA fragments were derived from putative promoter CGIs. Ten ovarian cancer cell lines were ana-

lyzed by methylation-specific PCR, and seven (*GPR150*, *LOC222171*, *PRTFDC1*, *LOC339210*, *ITGA8*, *C9orf64* and *HOXD11*) of the 33 CGIs were methylated in one or more of the cell lines. Expression of downstream genes of those methylated CGIs were analyzed by quantitative reverse-transcription-PCR and the result showed that those genes were not expressed in cell lines without unmethylated DNA. Demethylation of methylated cell lines with 5-aza-2'-deoxycytidine restored expression of two genes (*PRTFDC1* and *C9orf64*). In primary ovarian cancers, CGIs of *GPR150* (in 4 of 15 cancers), *ITGA8* (2/15), *PRTFDC1* (1/15), and *HOXD11* (1/15) were methylated. Silencing of *PRTFDC1* was revealed that aberrant methylation of *GPR150*, *ITGA8* and *HOXD11* could be candidate as tumor markers.

#### P04.062

##### The study of P53 gene mutations in patients of breast cancer in Rafsanjan city

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Background: P53 gene Mutation is the most common genetic change in human neoplasia. In breast Cancer, p53 mutation is associated with more aggressive disease and worse overall survival. The PCR-SSCP is the common test for mutation analysis of p53.

Materials and methods: DNA extraction from 48 paraffin Tissue samples of patients done by standard Phenol chloroform method, exons 5-8 amplified by PCR and PCR products underwent SSCP gel analysis for detection of probable mutations.

Results: Abnormal movement of PCR products band in SSCP gel that stained with silver nitrate reported as mutation. We found three mutations in exon 5, 2 in exon 6, one in exon 7 and 2 in exon 8.

Discussion: 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 or breast cancer will require long-term follow-up studies.

#### P04.063

##### p53, p63 and p73 isoforms in breast cancer

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The spectrum of genetic alterations identified in cancer cells includes mutations in various genes leading to structural and functional dysfunctions in signal transmission as well as over-or under expression of positive or negative signal generating proteins. Recently, two family members of the suppressor gene p53 have been described, p63 and p73, which seem to be necessary for specific p53-induced stress-response pathways.

Furthermore, p63 and p73 appears to be crucial to determine the cellular sensitivity to anticancer drugs, particularly in tumors lacking functional p53.

For analysis we used invasive breast carcinoma of common types with a different differentiation and stages as well as a normal breast tissue from patients with benign and malignant tumors. Protein p53 expression was estimated by Western blot analysis using anti p53 Abs DO-7 and CM-1. Our data show that breast cancer cells express nine different isoforms of p53, p63 and p73. The changes in the interactions between p53,p63 and p73 isoforms are likely to be fundamental to our understanding in the transition between normal cell cycling and the onset of tumor formation.

Therefore, determination of p53 status in clinical studies is much more complex than hitherto appreciated. It suggests that it requires an integrated and complex analysis of p53 isoform expressions associated with p53 mutation analysis and immunohistichemistry. To date, no clinical studies have integrated all those p53 parameters to determine p53 status.

**P04.064****Dissection of allelic molecular pathology of the SEMA6B gene frequently altered in breast cancer**

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We have assessed promoter methylation, altered expression and allelic imbalance of the SEMA6B gene in sporadic breast cancer and report that at least one of these molecular alterations is found in 75% of tumors. Taken separately, allelic imbalance was demonstrated in a half of all tumor samples, reduced expression and abnormal promoter methylation in 40% each. At the same time, the features supposed to be in close relation to each other, such as allelic imbalance and expression and/or promoter methylation and expression were poorly correlated. Several samples demonstrated intact gene expression in the presence of methylation and/or allelic imbalance and vice versa, questioning functional interactions between these parameters. Similar observations were made for several other candidate tumor suppressor genes, including another member of the semaphorin family, SEMA3B. We suggest that this may be a result of a nonallelic approach, where each form of molecular pathology is analyzed in the sample as a whole, without allelic discrimination. We have developed assays to assess allelic alterations based on a minisequencing technique in its SnaPshot modification (Applied Biosystems, USA). Having elaborated a coding intragenic SNP rs2304213 with heterozygosity close to 50% we have evaluated allelic imbalance and patterns of allelic methylation and gene expression in 22 informative samples of breast cancer versus adjacent control tissues. Data obtained from each individual sample will be presented. *The study is supported by Friends for an Earlier Breast Cancer Test Foundation, USA.*

**P04.065****Mutation screening of BRCA1 using Non-Radioactive PCR-SSCP analysis at 17q21 in breast carcinomas from non-familial cases**

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BRCA1 is a well-established breast cancer susceptibility gene. Correlation of breast cancer with BRCA1 mutation was studied in sporadic breast cancers since this gene is implicated in the double strand DNA repair and mitotic checkpoint, and loss of its function is speculated to result in the accumulation of chromosomal instability. In the present study, breast tumors of sporadic cancer were investigated for allelic imbalance (AI) at the BRCA1 locus. Furthermore, 30 breast carcinomas from patients with sporadic disease were examined to detect BRCA1-mutation(s), including exons 5, 11A, and 11B by using non-radioactive polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) technique followed by direct sequencing. Designed primers were used to amplify three DNA fragments of 235, 300, and 296 bp for detection of these exons mutations, respectively. After the PCR products were denatured, we have used SSCP technique to detect BRCA1 mutations. We investigated mutations in 6 cases (20%); 4 cases (13.3%) in exon 5, no case in exon 11A and 2 cases (6.7%) in exon 11B. Clinicopathological patients information was obtained from their files and pathologic reports. The relationship between these exons mutations and the clinicopathological variables was analyzed by the Fisher's exact test. Our results suggest that exon 5 and exon 11B gene mutations contribute to the development of sporadic breast cancer in kermanshah people.

**P04.066****Investigation of chromosomal radiosensitivity in the peripheral blood of Iranian women with sporadic Breast cancer: A pilot study**

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Breast cancer is the most frequent malignancy and the main cause of death amongst women worldwide and in Iran. Links between cancer predisposition and cellular radiosensitivity are well established and arose from investigations into chromosomal instability syndromes such as ataxia-Telangiectasia.

It has been shown that sensitivity to ionizing radiation by induction of chromosome aberration is, on average, higher in lymphocytes of breast cancer patients compared to healthy controls. It is therefore important to show that elevated chromosomal radiosensitivity may be a marker for breast cancer predisposition. In addition to chromosomal instability syndromes, approximately 40% of patients with breast cancer, 30% with colorectal cancer and 30% with head and neck cancer appear to show evidence of cellular radiosensitivity compared to healthy controls using the chromosomal G2 assay. Approximately 5-14% of the normal population has been shown to be radiosensitive and the connection between predisposition to cancer and radiosensitivity has led to the suggestion that chromosomal radiosensitivity may be used as an indicator of cancer proneness in the normal population.

In this study, 32 patients with sporadic breast cancer and 30 normal women as controls were investigated for their Chromosome radiosensitivity using gama irradiation for the cultured lymphocytes at G2 stage of the cell cycle. Our results showed significant increase in chromosome breakage in the patients compared to normal controls. There was also heterogeneity in chromosome breakage rate within the normal controls, which may suggest an increased risk for breast cancer in some normal women with increased chromosome breakage.

**P04.067****X Inactivation analysis in BRCA1 carriers and in patients with hereditary breast cancer**

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One of the two X chromosomes in female mammalian cells is inactivated in early embryonic life. The majority of females have a 50:50 distribution of the two cell types. A deviation from this distribution (skewed X inactivation) occur in female carriers of some X linked disorders. Furthermore, recently it has been reported that early onset breast cancer (BC) patients and BRCA1 carriers have a significant higher frequency of skewed X inactivation than controls.

The aim of this work was to compare X inactivation in two groups of BC women: BRCA1-carriers and BRCA1 negative cases with familial BC. We have studied 14 women carrying a BRCA1 pathogenic mutation (median 40.6 years old); 48 women with non-BRCA1/BRCA2 mutation (median 46.1 years) and a high familial history of BC ( $\geq 3$  close relatives with BC). We also studied a control population of 56 women without BC and with no history of X-linked pathologies (median 41.9 years).

DNA was extracted from peripheral blood and X inactivation pattern was determined by PCR analysis of a polymorphic CAG repeat in the first exon of the androgen receptor gene (AR).

One hundred and three women were heterozygous for the CAG repeat (103/118=96%). Skewed X inactivation appeared only in 1 woman of the control population (1/50=2%) and in none of the BC women.

Data presented in this study does not support any association between BRCA1 or breast cancer in inactive X heterochromatinisation, although this interpretation could be limited by the sample size.

**P04.068****In Silico analysis of BRCA1 and BRCA2 sequence variants of unknown clinical significance: application to variants found in healthy women in Croatia**

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BRCA1 and BRCA2 are the major hereditary breast and/or ovarian cancer predisposing genes and their mutations increase the risk of developing cancer. At present, almost half of all BRCA1 and BRCA2 sequence variants found are unclassified variants (UVs) so their clinical significance is unknown or uncertain. That represents problem for risk assessment in genetic counselling. After revealing BRCA1 and BRCA2 sequence variants in healthy Croatian females, our aim was to find fast in silico method for assessing preliminary clinical significance

of UVs newly found in patients.

We used different publicly available programs and web-based tools to identify UVs that may have deleterious effects with respect to different biomolecular functional categories (splicing regulation, transcriptional regulation, nonsynonymous amino acid SNP effect...) so their clinical significance in cancer etiology could be assumed.

Using straightforward physical and comparative considerations, we have found that several sequence variants with nonsynonymous amino acid change could have possible impact on the structure and function of a BRCA1 and BRCA2 proteins. Synonymous amino acid changes (silent mutations) could have impact on splicing regulation by disrupting exonic splice enhancers. Intronic sequence variants showed no potential impact on splicing because nucleotide changes at that positions likely make no changes in consensus splice sites.

In Silico analysis of BRCA1 and BRCA2 presents fast, easy and cheap method for assessing preliminary clinical significance for UVs, especially in cases with low frequency and ethnic specific alleles, when it is difficult to make population based studies and when expensive experimental functional assays must be performed.

#### P04.069

#### Three years of real-time PCR-based gene dosage for *BRCA1* large rearrangements in France : new perspective in combination with HRM curve analysis

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*BRCA1* germline mutations are responsible for susceptibility in breast-ovarian cancers. Some alterations are large rearrangements (15%). To detect them, our laboratory has proposed a real-time PCR-based gene dosage. Since 2004, samples from all of over France have been analyzed with this method to confirm large rearrangements detected with semi-quantitative multiplex PCR (MLPA or QMPSF). Recently, our qPCR experience have been transfer to LightCycler 480 combining High-Resolution Melting curve analysis.

For each genomic DNAs sent, suspected exons were scanned with a few others. Copy numbers of each exon were calculated with an algorithm based on delta Ct method. As reference of the diploidy, three genes (4q, 8q, 17q) were quantified.

148 samples have been analysed with ABI Prism 7900 and PowerSybr Mix. 49 rearrangements were confirmed (table). 99 samples have no rearrangement. Most of those *BRCA1* large rearrangements were analysed and validated with LightCycler 480 and HRM Master Mix using the same qPCR primers. Furthermore, by applying the same algorithm and PCR conditions, selected HRM primers were also able to detect those rearrangements.

This large series confirms the abilities of qPCR to detect large rearrangements in a routine screening. HRM curve analysis combined with quantitative PCR can give in unique assay information for point mutations and large rearrangements. Therefore, this technique should be reconsidered as a first-line tool for *BRCA1* screening.

Result of 148 suspected *BRCA1* large rearrangements by real-time PCR-based gene dosage

	Number of samples	Number of confirmation	%
Deletion suspected	101	35	35%
Duplication suspected (5'region to exon 1-2)	16	4	25%
Duplication suspected (others exons)	31	10	30%

#### P04.070

#### Characterization of some acute lymphoblastic leukemia cases by cytogenetic technique

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Cytogenetical analyses are important for diagnostics, prognostic and monitoring of the treatment efficiency in leukemia.

#### Objective:

Cytogenetical characterization of some Romanian patients with acute lymphoblastic leukemia (ALL).

#### Material and methods:

Five patients with ALL from Fundeni hospital, Bucharest were analyzed.

Bone marrow cells were cultivated in specific medium for 96 hours. The slides were prepared according to the classical procedure and stained with Giemsa solution. 100 metaphases/patient were analyzed.

The cytogenetic analyses follow two directions:

- detection of chromosomal aberrations and identification of t(9;22)
- evaluation of a potential link between leukemic phenotype and the telomeres structure.

#### Results and conclusion:

An obvious genomic instability of the leukemic cells was noticed, expressed by different chromosomal rearrangements, as well as by genomic mutation i.e. aneuploidy (table).

Patients	Normal metaphases	Chromosomal rearrangements				
		PCD	Aneuploidy (>50chromosomes)	Telomere-to-telomere and telomere-to-centromere attractions	Fusions	ring chromosomes
1	43	10	1	8	12	0
2	55	15	3	2	7	1
3	29	11	0	1	5	0
4	48	13	8	3	3	2
5	35	20	5	7	7	0

The FISH analyses showed the t(9;22) was present in two patients. This translocation was present in 57% of the metaphases of the first patient and in 71% of the metaphases of the third one.

No correlation between the telomere length and the leukemic phenotype could be detected. The fluorescence signal was encountered in more than 97% of the chromosomes, that indicates almost intact telomeric repetition, without large terminal deletions.

The incidence of t(9;22) found in two out of five patients is comparable to other studies taking in account also that they didn't receive any treatment, being at the diagnostic stage.

#### P04.071

#### Chromosomes 3 and 7 rearrangements in complex chromosomal aberrations in patients with myelodysplastic syndromes and acute myeloid leukemia

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Rearrangements of chromosomes 3 and 7 are frequent in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) and are associated with poor outcome and resistance to treatment. Combinations of molecular cytogenetic techniques were used for identification of these rearrangements in bone marrow cells of patients with complex chromosomal aberrations (CCA). In cohort of 392 patients with AML and RAEBt (refractory anemia with excess blast in transformation) we proved CCA in 58 of them. Chromosome 3 was involved in complex aberrations in 26 patients, chromosome 7 in 23 patients, both chromosomes 3 and 7 in nine patients. The most frequent aberrations of chromosome 3 were non-reciprocal translocation combined with deletion of chromosome 3 (14 patients). Other abnormalities proved by FISH: non-reciprocal translocations without deletion (8 patients), reciprocal translocation (5 patients), partial trisomy (4 patients), inversion (1 patient) and insertion of a part of chromosome 3 into chromosome 2 (1 patient). Breakpoints 3p24.2 and 3q26 were the most frequent. Minimal deleted segment 3p25-3pter was ascertained in 12 patients. Similarly, large heterogeneity of the breakpoints and various extent of deletion were proved on chromosome 7 with the most frequent breakpoints 7q31 and 7p12. Partners in translocations were chromosomes 5, 3 and 12. Using combination of molecular cytogenetic techniques we found a wide variety of aberrations not detectable by conventional cytogenetic analysis. We presume contribution of more genes and participation of epigenetic factors in the origin and during the course of the disease.

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**P04.072****Acute promyelocytic leukemia relapsing as secondary acute myelogenous leukemia with t(3;21)(q26;q22): molecular characterization and clinical follow-up in a patient with AME fusion transcript and review of the literature**

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Acute promyelocytic leukemia (APL) is a subtype of acute myelogenous leukemia (AML) that is characterized by peculiar clinical and biologic features such as severe hemorrhagic diathesis, specific chromosomal aberration with t(15;17)(q22;q12), and distinct morphologic features with predominant abnormal promyelocytes. In contrast to other subtypes of AML, APL has a particular sensitivity to treatment with all trans-retinoic acid (ATRA) combined with chemotherapy; converting this once frequently fatal leukemia to a highly curable disease. However, therapy-related myelodysplastic syndrome/acute myelogenous leukemia (t-MDS/AML) has been rarely reported as a late complication of chemotherapy in APL. To our knowledge, 30 APL cases have been described as t-MDS/AML in the literature. Among these reports, only one case relapsed as an acute leukemia with t(3;21)(q26;q22). Here we describe a 48-year-old female who was diagnosed with APL relapsing as a secondary AML with t(3;21). In this study, chromosome analysis as well as fluorescent *in situ* hybridization (FISH), multi-color FISH (mFISH), and reverse transcriptase-polymerase chain reaction (RT-PCR) for *AML1/MDS1/EVI1* (AME) fusion transcript were performed. Although allogenic peripheral blood stem cell transplantation (PBSCT) was done, bone marrow biopsy after PBSCT still revealed leukemic marrow in this patient.

**P04.073****Array-CGH analysis of Turkish adult ALL patients**

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In basic cytogenetic analysis 40-50% of acute lymphoblastic leukemia (ALL) patients show a normal karyotype. ALL is a clinically heterogeneous group, but cryptic copy number alterations could explain some of the heterogeneity. Array based comparative genomic hybridization (array-CGH) allows genome-wide high-resolution analysis of copy number alterations that are not detectable by standard methods including fluorescence *in situ* hybridization (FISH) or comparative genomic hybridization (CGH). There are reports of array-CGH studies on leukemia patients, but to our knowledge no published array-CGH studies exist on adult ALL. In order to assess the diagnostic yield of array-CGH on adult ALL, we performed pilot experiments on 14 adult ALL cases using catalog Agilent 44K oligonucleotide arrays. Eleven (78%) ALL patients had cryptic chromosomal aberrations. Deletions including the loci at 9p22.2 (n=2), 11p14.1 (n=2), 7p22.3 (n=2), and amplifications including the loci at 16p13.12 (n=2), 19q13.32 (n=2) are observed in at least two different patients. Our preliminary results indicate that array CGH analysis provide additional information about cryptic genetic aberrations during cytogenetic studies of adult ALL patients.

**P04.074****Der(7;10)(p10;q10) in acute myeloid leukemia**

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We report a case of a female patient with der(7;10)(q10;q10) resulting in loss of long arm of chromosome 7 that has an established prognostic value in hematological malignancies. In acute myeloblastic leukemia (AML) most often deletion (7q) and monosomy 7 are observed. Both changes are classified as poor prognostic criteria. Whole-arm translocations of chromosome 7 are rarely described. Translocation with the long arm of chromosome 1 occurs nonrandomly in myelodysplastic

syndrome and AML. While there have been reports on significantly better clinical outcome than other -7/7q cases the same rearrangement was also recognized as very poor prognostic feature.

In April 2007 a 23-year old woman was admitted to the hospital. Diagnosis workup confirmed myelo-monocytic leukemia (AML M4/M5). Cytogenetic examination of bone marrow cells revealed that one normal chromosome 7 and 10 were lost and replaced by an abnormal chromosome consisting of the long arm of chromosome 10 and the short arm of chromosome 7. Additionally, a small marker chromosome was observed in all analyzed metaphases. The whole-arm exchange was confirmed by FISH using WCP and CEP probes as well as a locus specific probe (7q32). Combining the results of standard and molecular cytogenetics the karyotype was interpreted as: 46,XX,der(7;10)(p10;q10),+mar. The marker chromosome was found to consist of the chromosome 7 centromeric material. The patient is in cytogenetic remission now. Her condition will be further followed to clarify the significance of this chromosomal change. According to our best knowledge it was not described in hematological malignancies before.

**P04.075****Gains of chromosome 2p in chronic lymphocytic leukemia**

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Chronic lymphocytic leukemia (CLL) is the most common adult leukemia. The progress in molecular genetic characterization of CLL confirmed the prognostic role of IgV<sub>H</sub> mutational status, expression of CD38, 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 (p53) detected routinely, the other additional abnormalities can be found. It is presently not clear whether other aberrations that are not detected by the standard FISH panel have any impact on prognosis and disease progression. Therefore, we performed clinical and laboratory analysis of 7 CLL patients (males) with detected gains of chromosome 2p. The aim of the genetic study was supported by the fact, that gains of genetic material can lead to oncogenic activation of protooncogenes located within the gained regions. Since 2p23-p11 harbors many protooncogenes already known to be involved in human tumorigenesis, we desided to check a few selected genes: ALK, N-MYC, REL, BCL11a and their protein levels.

Comparative genomic hybridization (CGH) or 1 Mb arrayCGH revealed gain of 2p23-p11 region. For detailed mapping of gained region we used iFISH with commercial available and BAC-derived probes. In all cases IgV<sub>H</sub> mutational pattern was established and immunohistochemical analysis of selected proteins was performed.

Our study will summarize molecular cytogenetic, molecular genetic and clinical findings with respect to the 2p abnormalities in 7 CLL patients.

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**P04.076****Cytogenetic study of variant acute promyelocytic leukemia**

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Hematologic malignancies are heterogeneous group of clonal hematopoietic stem cell disorders resulting from wide spectrum of nonrandom chromosome abnormalities ranging from balanced translocations to unbalanced karyotypes with numerical and/or structural gains or losses and to complex rearrangements involving three or more chromosome abnormalities. In >95% acute promyelocytic leukemia (APL), t(15;17) is the hallmark of pathogenesis while remaining cases have cryptic insertion or more complex rearrangements where *RARA* is constantly involved. The fusion of *PML-RARA* disrupts the normal interaction of retinoic acid and *RARA*, and converts *RARA* into a transcription activator. All-trans retinoic acid receptor (ATRA) is able to interact with this mechanism and prevent life-threatening thromboembolic/bleeding complications. We have investigated 30 APL patients by conventional G-banding and detected two (7%) cases with t(11;17)(q23;q22), which leads to fusion of *PLZF*(promyelocytic leukemia zinc finger) and *RARA*

genes. One of them had two additional unbalanced translocations, t(12;14)(p;q11) and t(12;16)(p;p) with complex karyotype such as 45,XY,-14,t(11;17)(q23;q21),der(12)t(12;14)(p11;q11),der(16)t(12;16)(p11;q13) in 96% cells. It has been reported that such variant cases are resistant to ATRA and exhibit no therapeutic response, though resemble *PML-RARA* morphologically. Therefore, these two patients are presumed to experience poor prognosis. Investigation of all these cases by FISH for *PML-RARA* would have given normal result to these two patients and could have led to progression of the disease condition. Therefore, it's understood that conventional cytogenetics still plays an important role in diagnosis, classification, prognostication and monitoring of hematologic malignancies in the era of microarray technology and cannot be replaced by any other molecular technique.

#### P04.077

##### Cytogenetic study of chronic myeloid leukemia (CML)

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To explore the clinical cytogenetic feature & prognosis of chronic myeloid leukemia patient, evaluation of 8 CML cases (6 men and 2 women) from the Indian population with ages ranging from 25 to 55 years were studied in the present dissertation project. Bone marrow samples were processed following direct method &/or 24hrs culture without mitogen for chromosome preparation and karyotype analysis of G-banded metaphases. 25 metaphases were studied for each case using karyotyping software (Metasystems, Germany). All 8 cases showed Philadelphia chromosome i.e. t(9;22)(q34;q11) in 100% cells. Two (25%) cases showed extra Philadelphia chromosome in 8% & 38% cells respectively. One case had missing of Y chromosome in addition to Philadelphia chromosome. It has been established that the major secondary chromosomal aberration in adult is double Philadelphia chromosome & minor one is -Y chromosome. The i(17)q is not very common in adult. Since chromosome 17 was normal in all the above cases the prognosis of these patients will be comparatively better. However, the prognosis of patient in which abnormal metaphase with +ph chromosome was 38% expected to be poor compared to the patient with 8.6% abnormal metaphases. Due to the additional chromosomal abnormality, patient may acquire resistance to Imatinib - the tyrosine kinase inhibitor. Therefore, it is understood that cytogenetic study is important in diagnosis & prognostication of CML patient.

#### P04.078

##### Another case with der(8)t(8;17)(p11;q11) in Sézary's syndrome

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Sézary's syndrome (SS) is a peripheral T-cell neoplasm characterized by a pruritic exfoliative or infiltrated erythroderma, lymphadenopathies, and atypical T lymphocytes in the peripheral blood. Chromosome abnormalities, mostly complex karyotypes, are seen in about 50% of patients with SS, and there have only been a few instances of recurrent rearrangements.

We report a 60 year-old female referred to Hematology Service due to 20 months history of lymphocytosis. The patient showed a cutaneous hyperpigmentation and bilateral axillary and inguinal lymphadenopathies. The peripheral blood smear presented 40% of Sézary cells. The diagnosis was Mycosis Fungoide with leukemic involvement.

The karyotype in peripheral blood was performed. We used G-banded technique in conventional cytogenetics. Molecular cytogenetics (FISH analysis) permitted discovering the rearrangements involved.

The whole chromosome paints (WCPs) for chromosomes 1, 5, 9, 10, 11, 15, 17 and 19 were used. Finally the karyotype was: 44-45, XX, t(1;5)(p33;q31)[4], der(8)t(8;17)(p11;q11)[4], -9[2], inv(9)(p21;q34)[2], -10[4], del(11)(q21)[4], der(14)t(10;14)(?q?;p11)[4], del(15)(q22)[4], der(15)t(9;15)(q21;q26)[2], -17[4], +mar1[4] [cp4] / 45, X, -X[6] / 46,XX[10].

Cytogenetic alterations were concordant with other abnormalities described before in SS, like: breakpoints at 1p32-36 and 10q23-26, and other alterations involving chromosomes 8, 9, 11 and 17. But the most important event was the presence of der(8)t(8;17)(p11;q11), because to our knowledge, this is the fourth case with the same alteration re-

ported in SS. First of all was Thangavelu M, et al. Blood (1997) with two cases with der(8)t(8;17)(p11;q11) and a psu dic(17;8)(p11.2;p11.2) was observed by Batista D, et al. Genes, Chromosomes and Cancer (2006).

#### P04.079

##### Der(1;16)(q10;p10) in Acute Myeloid Leukemia: the first female case described

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Whole-arm translocation of chromosome 1 and 16 is recurrently observed in non-hematologic neoplasias such as breast carcinomas and Ewings sarcoma but rarely in hematologic neoplasias, mostly in multiple myelomas. To our knowledge, only 7 cases were described in MDS/AML, interesting all were males.

Here we describe the first female patient with AML M4/M5 with der(1;16)(q10;p10) as the sole chromosome rearrangement found.

Cytogenetic examination of bone marrow cells revealed that the normal chromosome 16 was lost and replaced by an abnormal chromosome consisting of the long arm of chromosome 1 and the short arm of chromosome 16, resulting in trisomy of the long arm of chromosome 1 and monosomy of the long arm of chromosome 16. The karyotype was confirmed by FISH: 46,XX,+1,der.ish (1;16)(q10;p10)(wcp1+,wcp16+,cep16+).

Due to a small number of metaphases available and bad resolution of chromosomes, CGH was also performed. Only the amplification of the long arm of chromosome 1 and deletion of the long arm of chromosome 16 was confirmed.

Unfavorable outcome, including no response to chemotherapy, and short survival were characteristic of our patient. Despite very short survival of our patient, prognostic significance remains to be determined. It is possible that some other unidentified molecular mechanism could play a role in bad prognosis or simply the fact that this is the only female patient described until now. From this point of view, the pathogenesis of der(1;16) still remains unresolved.

#### P04.080

##### Detection of gene expression profile for BAALC and WT1 genes by real time RT-PCR in patients with acute myeloid leukemia

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Acute myeloid leukemia (AML) is a heterogeneous group of diseases characterized by uncontrolled proliferation of clonal neoplastic hematopoietic precursor cells and impaired production of normal hematopoiesis leading to neutropenia, anemia, and thrombocytopenia.

High mRNA expression of BAALC and WT1 have been shown to be an adverse risk factor in newly diagnosed AML patients with normal cytogenetics.

Independent confirmation is required to validate the initial results so that BAALC and WT1 expression may be exploited for risk-adapted treatment stratification of AML patients with normal cytogenetics.

We analyzed patients selected from newly diagnosed cases of AML and a control group of healthy individuals.

Total RNA from leukocytes was extracted from blood samples treated with RNA-later (QIAGEN) following the manufacturer's directions (RiboPure - blood kit, Ambion). For semiquantitative RT-PCR we used One-step RT-PCR kit (QIAGEN), with gene-specific, intron spanning primers for BAALC,WT1 and GPDH as housekeeping gene. Comparative real-time RT-PCR assays were performed for each sample in triplicate.

The results showed a significantly increased level of expression for BAALC and WT1 genes in de novo acute myeloid leukemia patients with normal cytogenetics comparative with healthy individuals from the control group.

In conclusion, the analyse of BAALC and WT1 genes expression in AML patients is a molecular marker which can be used for the evaluation of disease aggressiveness and prognosis for patients with normal cytogenetics.

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**P04.081****Discordance between morphological and cytogenetical remission in a patient with AML and t(2;11)(p21;q23)**

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We report here on a 58 year old male presenting with biphenotypical acute myeloblastic leukemia. Conventional cytogenetic techniques on bone marrow at initial diagnosis, before treatment, showed the following karyotype:

46,XY,t(2;11)(p21;q23), del(5)(q21), add(12)(p12)[9]/46,XY,t(2;11)(p21;q23)[5]/46,XY[1].

FISH analysis with a DNA probe for the MLL-gene demonstrated that the breakpoint at 11q23 was telomeric to the MLL gene. T(2;11)(p21;q23) is a rare but recurrent translocation observed in MDS and AML. This translocation is specifically associated with a deletion of the long arm of chromosome 5. After induction treatment the patient was in complete morphological remission. However, cytogenetic studies revealed the t(2;11) in all metaphases, without the del(5q) and the add(12p). Evaluation after the second therapy cycle showed again a complete morphological remission but persistence of t(2;11) in all analyzed metaphases. To investigate the possibility of a constitutional chromosome aberration chromosomal analysis was performed on skin-fibroblasts and peripheral blood. The skin-fibroblasts revealed a normal karyotype in all 100 analyzed metaphases. PHA stimulated blood showed a mosaic karyotype : 46,XY,t(2;11)(p21;q23)[11]/46,XY[21]. This suggests that the t(2;11) is an acquired aberration that persists in spite of morphological remission.

The patient underwent a bone marrow transplantation and is still in complete morphological remission after 10 months.

To our knowledge this is the first description of an AML case with a t(2;11) showing discordant morphological and cytogenetical remission.

**P04.082****A pediatric case with atypical CML with ETV6/PDGFRB fusion gene**

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Atypical chronic myelogenous leukemia (aCML) is a leukemic disorder that exhibits both myelodysplastic and myeloproliferative features at the time of diagnosis. The median age of this rare disorder has been reported to be in the seventh or eighth decade of life. Herein we report a rare pediatric case of a 14 months old boy who was presented with leukocytosis and frequent infections since the age of 6 months. Bone marrow showed marked myeloid hyperplasia with elements of dysplasia, particularly in eosinophil series. Conventional and molecular cytogenetic analysis of BM were performed. FISH analysis for chromosome abnormalities, commonly observed, in MDS/MPD were performed using the appropriate probes, such as LSI BCR/ABL, CEP 8, LSI CBFB, LSI D205108 and LSI D7S486/CEP7 (Vysis). FISH results revealed normal hybridization pattern for all the chromosomes analysed. Conventional cytogenetic analysis revealed a clone of 46,XY,t(5;12)(q33;p13). RT-PCR analysis for the detection of the fusion gene ETV6/PDGFRB was positive. In addition, FISH analysis using the ETV6 (12p13) split probe (Vysis), confirmed the translocation t(5;12)(q33;p13). With the above findings patient was diagnosed as aCML with Philadelphia chromosome negative and BCR/ABL fusion gene negative. The patient was treated with Gleevec (imatinib) for 8 months, achieved complete hematological and cytogenetic remission (MRD of 0.4% in the latest sample) and underwent MUD bone marrow transplantation.

**P04.083****BCR/ABL negative myeloproliferative neoplasms- cytogenetic and molecular study**

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of Virology, Bucharest, Romania, <sup>4</sup>"Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania, <sup>5</sup>Coltea Clinical Hospital, Bucharest, Romania. Myeloproliferative neoplasms (MPNs) originate in hematopoietic stem cell compartment and exhibit a consistent phenotypic diversity. Only a small fraction of patients harbour cytogenetic abnormalities (+8, +9, 20q-, 13q-, 12p-). The recent identification of JAK2 V617F mutation as a key genetic event in a majority of cases has greatly advanced the understanding of pathogenetic mechanisms in BCR/ABL negative MPNs.

Our study has been focused on cytogenetic and molecular characterization of BCR/ABL negative MPNs, with emphasis on correlation between cytogenetic changes and JAK2 mutation status.

Chromosome analysis, FISH and RT-PCR (Revers-Transcription PCR) have been used to investigate the BCR/ABL fusion and MPN-associated genomic reccurrent abnormalities in 28 patients by date, diagnosed with MPNs. Chromosome analysis was performed on cultured bone marrow cells (GTG banding). To detect cryptic chromosomal changes (13q14, 17p13.1, 20q12-13 deletions) interphase and metaphase FISH with various LSI probes (Vysis) and Microsatellite PCR (D20S206 and D20S119 Markers) were performed. Allele-specific PCR have been used to detect the JAK2 V617F mutation.

All 28 patients displayed BCR/ABL negative status. The JAK2 V617F mutation has been detected in 16 patients. The cytogenetic analysis revealed chromosomal anomalies in 4 patients (trisomy 8, complex cytogenetic changes). No loses at investigated loci were found (13q14, 17p13.1). All patients were heterozygous for at least one of the two DNA markers.

Further investigations will be performed in order to reveal other genetic abnormalities, thus, we hope to contribute both in more precise diagnosis and novel, patient-tailored therapeutic strategies.

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**P04.084****Microhomologies and interspersed repeat elements at genomic breakpoints in Chronic Myeloid Leukemia**

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Reciprocal translocation t(9;22) is central to the pathogenesis of chronic myeloid leukemia. Some authors have suggested that *Alu* repeats facilitate this process, but supporting analyses have been sparse and often anecdotal. The purpose of this study is to analyze the local structure of t(9;22) translocations, and asses the relevance of interspersed repeat elements at breakpoints. Collected data have been further compared to the current models of DNA recombination, in particular the Single-Strand Annealing (SSA) and the Non-Homologous End Joining (NHEJ) processes. We proposed a protocol for the rapid characterization of patient-specific genomic junctions, and we considered a total of 27 patients diagnosed with chronic myeloid leukemia. Sequences analysis reveals microhomologies at the junctions of 21 patients of 27, while interspersed repeats were of relevance ( $p<0.05$ ) in at least 16 patients of 27. These findings are more frequent than expected and give indication that the main mechanisms involved in the t(9;22) translocation are the SSA and NHEJ pathways, both playing a role. More in detail, our report is consistent with microhomologies facilitating the joining of DNA ends in the translocation process, and with both *Alu*, and a variety of other repeat sequences pairing non-homologous chromosomes during the SSA pathway

**P04.085****The four and a half LIM domain protein 2 (FHL2) interacts with CALM and is highly expressed in AML with complex aberrant karyotypes**

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The CALM/AF10 translocation t(10;11)(p13;q14) is found in acute myeloid leukemia (AML), T-cell acute lymphoblastic leukemia (T-ALL) and malignant lymphoma. The CALM/AF10 fusion gene has been shown to cause biphenotypic leukemia in a murine bone marrow transplant model. The CALM (Clathrin Assembly Lymphoid Myeloid leukemia

gene) protein is a clathrin assembly protein which plays a role in clathrin-mediated endocytosis and trans Golgi network trafficking. AF10 is a putative transcription factor likely involved in processes related to chromatin organization.

To learn about the function of CALM/AF10 fusion protein, we searched for protein interaction partners of CALM using a yeast-two-hybrid assay and identified FHL2 as a putative CALM interacting partner. The CALM-FHL2 interaction was confirmed by GST pull-down and CoIP experiments. In co-localization studies a translocation from cytoplasm to the nucleus is seen.

Expression analysis (Affymetrix based) in CML and different AML subtypes showed a significantly higher expression of FHL2 in CML and AML with complex aberrant karyotypes compared to AML with normal karyotypes or balanced chromosomal translocations like the t(8;21), inv(16) or t(15;17).

Reporter gene assays using a GAL4-DNA binding domain FHL2 fusion protein and a GAL4 responsive luciferase reporter were able to demonstrate a transcriptional activation function of FHL2 that got inhibited when adding CALM but not CALM/AF10. Previous findings show that high expression of FHL2 in breast cancer associate with an adverse prognosis.

It is thus conceivable that CALM/AF10 may play a role in a pathway that enforces progression of malignancy when not downregulating the FHL2 expression.

#### P04.086

##### **Polymorphisms and haplotypes of the NBS1 gene in childhood acute leukemia**

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DNA repair gene polymorphisms and mutations may influence cancer risk. The product of NBS1 gene, nibrin, is functionally involved in double strand DNA break repair system. Heterozygous, germline mutations of the NBS1 gene are associated with increased risk of tumors including familial/sporadic breast and prostate cancer, larynx cancer and childhood acute lymphoblastic leukemia. So far reports on NBS1 polymorphisms in lymphoproliferative diseases are scarce. The aim of the present study was to answer the question whether polymorphisms of NBS1 gene may influence susceptibility to the development of childhood acute leukemia. We genotyped c.102G>A, c.553G>C, c.1124+18C>T, c.1197T>C c.2016A>G c.2071-30A>T polymorphisms of the NBS1 gene in 157 cases of childhood acute leukemia and 275 control subjects.. The distribution of allele, genotype and haplotype of the polymorphisms were compared between cases and controls using PCR-SSCP and Chi-square test. The TT genotype of c.2071-30A>T polymorphism was increased in leukemia patients than in healthy controls ((p=0.04), OR=1.828 (1.005 to 3.325)). No significant differences in allele and genotypes frequencies at the other five polymorphisms sites were observed in a comparison of leukemia cases and controls. Genotyping data from six polymorphisms loci in NBS1 in leukemia cases and controls, were used to impute haplotypes. Three main haplotypes made up the majority of cases and controls (GGCTAA (41%), ACTCGT (20%), GGCCAA (11%)). Two of them GGCTAA and ACTCGT were associated with significantly increased leukemia risk, p=0.0038 and p<0.0001, respectively. Our results suggested that some specific haplotypes of the NBS1 gene may be associated with childhood leukemia cases.

#### P04.087

##### **Chronic lymphocytic leukemia: optimization of cytogenetic method for detection of chromosomal alterations**

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B-cell chronic lymphocytic leukemia (CLL) is the most frequent type of leukemia in adults in the Western world and is generally considered to be a disease of the older population. The role of cytogenetics in diagnosis and follow-up studies of CLL is now widely recognized. According to the literature, clonal chromosome aberrations are detected in approximately 40-50% of tumors. However, conventional cytogenetic analysis is not currently applied to CLL's study, due to the low mitotic activity of the tumor cells *in vitro*.

The aim of this study was describe a reliable cytogenetic technique for the detection of chromosomal alterations in CLL. We have tested several protocols described in the literature and optimized them in order to obtain metaphases in all samples.

We describe the methodology and discuss the results according to the different protocols applied.

#### P04.088

##### **Residual disease monitoring in chronic myelogenous leukaemia (CML) using tricolour dual fusion fluorescence *in situ* hybridisation (FISH) probe system**

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**Objectives:** We investigate the application of the *BCR-ABL* + 9q34 tri-colour dual fusion translocation probe system (Vysis) for the monitoring of residual disease in chronic myelogenous leukaemia (CML) with der(9) deletion through analysis of both interphase and metaphase FISH signal patterns.

**Methods:** *BCR-ABL* dual colour dual fusion FISH (D-FISH) was performed on the diagnostic samples of 22 CML patients. The tri-colour system was tested on cases that showed aberrant signal patterns other than the classical 1G1O2F. Using the aqua band-pass filter, random signal overlap would be indicated by the presence of an aqua signal (ASS) in the "fusion" signal, while genuine fusion was represented by the absence of the ASS signal.

**Results:** Using the D-FISH system, the signal patterns could be categorized into 4 groups: group 1 (n = 17) showed the classical 1G1O2F; group 2 (n = 2) showed 2G1O1F indicating *ABL* deletion; group 3 (n = 1) showed 1G2O1F indicating *BCR* deletion; group 4 (n = 2) with 1G1O1F indicating reciprocal *ABL-BCR* deletion. The tri-colour dual fusion system showed excellent correlation with D-FISH for cases with der(9) deletion. In particular, the added aqua ASS probe allowed the discrimination of random signal overlap from genuine *BCR/ABL* fusion in the interphase cells (group 4).

**Conclusion:** Although the D-FISH probe remains valuable in establishing the different patterns of aberrant signals and monitoring patients with the classic 2-fusion signals in CML, the tri-colour dual fusion translocation probe should be used for patients with der(9) deletion to monitor response to treatment.

#### P04.089

##### **Clonal evolution and genomic instability in chronic myeloid leukemia during imatinib mesylate therapy**

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Chronic myeloid leukemia is characterized by the Philadelphia chromosome which originates in most cases from the translocation t(9;22)(q34;q11), and leads to the formation of *BCR/ABL* oncogene. Ph positive clonal evolution, including amplification/duplication of the *BCR-ABL* gene, has been found to contribute to disease progression and imatinib mesylate resistance.

We report the results of a study monitoring the dynamics of chromosomal anomalies in Philadelphia positive hematopoiesis during imatinib therapy.

Cultured bone marrow cells were used for chromosomal studies. FISH studies with *BCR/ABL* dual fusion probe and locus specific probes for various chromosomal loci were performed. The follow-up interval ranges from 6 to 60 months.

Imatinib therapy was monitored in 80 Ph positive CML patients. Seventy eight patients showed standard translocation t(9;22) and two, a variant Philadelphia translocation. Four patients demonstrated additional chromosomal abnormalities, at diagnosis. Duplication of the *BCR-ABL* gene as free additional der(22) or isochromosome ider(22)t(9;22) was observed in 4 out of 6 cases with clonal evolution. Three cases showed unusual additional changes (translocations, additions). An unexpected sequence of events leading to accumulation of multiple aberrations [ider(22)t(9;22);i(17)(q10);+8] was observed in one case.

Clonal cytogenetic aberrations of Ph positive hematopoiesis is considered to herald disease progression and to reflect the genomic instabil-

ity of the malignant clone in CML. We appreciate that all new cases might bring new insights, with a special emphasis on prognostic impact of additional chromosomal changes in imatinib therapy era.

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#### P04.090

##### Complex karyotype and cryptic t(9;22) translocation at the onset of chronic myeloid leukemia - case report

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Among patients with chronic myeloid leukemia (CML) positive for Philadelphia chromosome, 5-8% show variant translocations in which at least a third chromosome in addition to 9q34 and 22q11 is involved. We report an apparently Ph negative CML patient with unusual complex karyotype showing a typical fusion transcript detected by reverse transcription PCR (RT-PCR) and BCR/ABL fusion gene localized in a complex rearranged chromosome identified by FISH.

Cell cultures from bone marrow and peripheral blood, and GTG banding were performed for karyotype investigation. Dual fusion BCR/ABL probe, chromosome painting (WCP 9 and WCP 22) and locus specific BAC (1p36.33 and 17p11.2) probes for metaphase FISH analysis were used. RT-PCR for BCR/ABL fusion transcripts detection was applied. Bone marrow cytogenetic analysis showed a complex rearrangement consisting of chromosome 1 deletion, 17p addition, telomere-to-telomere fusion of chromosomes 21 and 22, but no standard Philadelphia translocation. Constitutional aberrations were ruled out by peripheral blood karyotyping. RT-PCR detected BCR/ABL fusion transcript. FISH analysis showed two fusion signals for hybrid gene, and confirmed the complexity of rearrangements involving chromosomes 1, 9, 17, 21 and 22.

The FISH techniques allowed us the identification of chromosome rearrangements that could not otherwise be detected by conventional banding procedures.

The frequency, formation mechanisms and clinical significance of such rare type of clonal rearrangements need to be investigated.

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#### P04.091

##### Major causes of imatinib resistance in chronic myelogenous leukemia: BCR-ABL kinase domain mutations and clonal evolution

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Imatinib mesylate, a targeted BCR-ABL tyrosine kinase inhibitor (TKI) has become the standard drug for the treatment of chronic myelogenous leukemia (CML). Mutations in the kinase domain (KD) of BCR-ABL and nonrandom, karyotypic abnormalities (referred to as clonal evolution) contribute to imatinib-resistance. Imatinib-resistance may be defined as primary or secondary, hematologic or cytogenetic and it occurs in 10-15% of patients with CML. The aim of our study was to screen for BCR-ABL KD mutations and additional chromosomal abnormalities (ACA) in 35 Hungarian patients (33 CML and 2 Philadelphia chromosome positive ALL) with imatinib-resistance. Point mutations in the entire tyrosine kinase domain (aa 230-490) were detected by Sanger-sequencing after two separate, two step semi-nested PCR. The presence of T315I, M244V, Y253H, M351T, F359V, L384M mutations were confirmed by PCR-RFLP. Clonal alterations were assessed by standard cytogenetic techniques. Overall, twelve different mutations (aa. exchange M244V, G250E, Y253H, E255V, E279K, D276G, T315I, M351T, F359I/V, V379E, L384M, E460K) were identified in 21 patients. M244V was the most frequent mutation (4 patients). T315I occurred in

2 ALL and one CML patients. In three cases, double mutations, and in 8 patients, extra Philadelphia chromosome were detected. ACA was present overall in 13 patients. KD mutations were found in 75% (9/12) in hematologic, and 43% (9/21) in cytogenetic TKI resistance. In summary, BCR-ABL KD mutations and clonal evolution are common in imatinib-resistant CML patients. Early detection of emerging mutant clones may guide therapeutic decisions, because the degree of imatinib-resistance varies among different mutants.

#### P04.092

##### Mutation profile of BCR-ABL kinase domain in imatinib primary and secondary resistant chronic myeloid leukemia cases

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Chronic myeloid leukemia (CML) is hematopoietic stem cell disorder characterized by balanced translocation t(9;22). Fusion gene *BCR-ABL* appeared in the result of t(9;22) gives rise to Abl tyrosine kinase activation with followed leukemogenic effects. Bcr-Abl selective tyrosine kinase inhibitor imatinib mesylate revolutionized CML therapy and allow achieve complete cytogenetic remission (CCR) in most cases of chronic phase of CML. Nevertheless refractoriness (primary resistance) or relapse of initial response (secondary resistance) are observed in over 30% cases of CML. More than 40 different point mutations of *BCR-ABL* decrease sensitivity to imatinib. To study *BCR-ABL* mutations profile in imatinib resistant cases of CML we have studied *BCR-ABL* kinase domain mutations in 27 patients (middle age 43 y.o. from 21 to 60) with chronic phase of Ph+ CML that did not achieve any cytogenetic response (95-100% Ph+ BM cells) after 1 year imatinib therapy 400 mg daily (n=22) or loose cytogenetic response (n=5). Mutation status was studied by direct sequencing of *BCR-ABL* cDNA samples. *BCR-ABL* kinase domain mutations were founded in 7 patients (25,9%). The mutational spectrum included five missense mutations: M244V, L248V, Y253N, M351T, T315I. Five primary resistant cases were characterized by L248V mutation (3 cases), T315I (1) and M244V+M351T (1). Two patients with secondary resistance shown L248M (1) and Y253N (1) mutations. In conclusion, mutation analysis of primary refractoriness or relapse of initial response CML patients is very useful tool for changing of CML strategy therapy include imatinib dose escalation, new generation of BCR-ABL inhibitors, combination therapy and BMT.

#### P04.093

##### Differential expression pattern of *ITPA* gene in CML patients

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Genetic material in nucleus or mitochondria is the most significant intracellular target of damages that cells exposure every day. One of the most important of these damages is oxidative deamination of DNA and free nucleotides in the cell pool. Incorporation of deaminated nucleotides such as inosine triphosphate (ITP, dITP) into DNA or RNA can increase the frequency of base substitution mutation. It has been suggested that presence and accumulation of these rough nucleotides can lead to genetic and chromosomal instability which is the perquisite of different types of diseases or cancer. The evidences demonstrate the role of inosine triphosphate pyrophosphates (ITPase) encoded by *ITPA* gene, in protecting the cells by omitting the rough deaminated purines nucleotides of the cell pool. Chronic myelogenous leukemia (CML) is a type of cancer which is mainly characterized by the presence of Philadelphia chromosome. There are some reports about existence of several structural and numerical chromosome abnormalities in addition to Philadelphia chromosome in these patients. The objective of this study is to compare *ITPA* gene expression in CML patients versus normal samples to examine the possible dysfunction of *ITPA* gene activity as an important predisposing factor for genetic instability. Our results revealed different expression pattern of *ITPA* gene expression between two groups.

**P04.094****Grafting outcome prediction by optimizing the protocol for molecular monitoring of leukaemia patients**

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Introduction. Disease relapse represents the major cause of treatment failure after allogeneic hematopoietic stem cell transplantation (allo-HSCT). The aim of our study was to optimise the testing protocol for establishing the chimeric status of patients performing allo-HSCT. We compared the efficacy of two different multiplex STR-PCR kits, focusing on their sensitivity and sensibility.

Method. DNA samples, provided by 17 patients and their haploididentical donors were tested at various intervals in order to determine the chimeric status. The samples were analysed using two different commercial kits, each of them containing a set of 16 STRs (AmpFISTR Identifier, Applied Biosystems; PowerPlex 16 System, Promega). For each case, the most informative markers were selected and analysed in dynamics. Artificial mixtures prepared from recipients and their donors samples were analysed to set up reconstruction curves.

Results. Complete chimerism was detected for 15 patients, while 2 patients exhibited mixed profiles. Both commercial kits gave similar results concerning the chimeric status. The reconstruction curves showed a slightly higher sensitivity for the Identifier kit, which quantified more accurately the low concentrations of minor DNA component.

Conclusions. Our data indicate that both commercial STR-PCR kits were able to establish an accurate chimeric status. A kit selection should be performed in an objective manner, after analyzing its performances regarding the typing sensitivity and sensibility for artificial mixtures. Thus, precise monitoring of post-transplant hematopoietic chimeric status becomes of outmost interest for the clinical follow-up of leukaemia patients.

**P04.095****Tracking the natural history of leukaemic clone in MLL-driven childhood acute leukaemias**

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We and others have previously documented prenatal origin of paediatric acute lymphoblastic leukaemias (ALL) by demonstrating identical clonal changes in monozygotic twins/triplets and/or backtracking of the (pre)leukaemic clone to neonatal blood spots or cord blood of patients. We have also detected the cells of preleukaemic clone in the cord blood of healthy newborns never developing leukaemia. However, the evolution of definitive leukaemic clone remains largely unclear. Secondary ALL developing during leukaemia treatment represents a unique chance to follow this process. We analysed two cases of treatment-related ALL involving notorious oncogenic transcription factor MLL. In both patients, cells bearing MLL fusion (MLL/FOXO3A, MLL/MAML2) appeared in bone marrow long before the definitive leukaemic clone was identified: 20 and 24 months, respectively. Moreover, in both cases, FISH and/or qRT-PCR showed this fusion in a substantial proportion (10%-90%) of marrow cells with morphologically intact differentiation during the preleukaemic phase. In the MLL/FOXO3A case we were able to document its presence in lymphoid as well as myeloid lineage, thus indicating the fusion arose in a multipotent progenitor. In both cases, definitive lymphoblastic leukaemic clones arose only shortly before relapse. Comparison by SNP-array revealed a 10Mb region of amplification on 19q13.32 in one of the leukaemic samples, absent in the preleukaemic phase. Taken together, we document a striking sequence of events including covert protracted preleukaemic phase characterised by dominant MLL-fusion with intact differentiation and subsequent acquisition of secondary genetic abnormality (proven

in one case). These data provide unique insight into the MLL-driven leukaemogenesis.

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**P04.096****Amplification of hTERC and hTERT genes in leukemic cells detected by fluorescent in situ hybridisation (FISH)**

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The high level of genomic amplification of the human telomerase genes hTERC and hTERT, which maps to chromosome bands 3q26 and 5p15, was determined in different human cancer cells. We represent the results of the fluorescent in situ hybridisation (FISH) analysis with both locus specific probes on cell cultures from bone marrow of 35 patients with acute malignant blood disease. Among them 32 patients were with non-lymphoid and 3 with lymphoid type of malignancy. Bone marrow cells were first karyotyped by standard cytogenetic analysis. FISH revealed a low grade amplifications of hTERC gene at 4/35 patients and a low grade of the hTERT amplifications at 5/35 patients. The comparison of the karyotypes and the FISH results by each patient reveals low grade amplification of hTERC gene only at one patient with previously determined complex karyotype. We didn't confirm any amplification of hTERT gene. Our results, based on comparison of the results derived by FISH and karyotyping, show that the hTERC and the hTERT amplification are not so common in patients with malignant blood disease as in other types of cancers.

**P04.097****MRP1 polymorphisms (T2684C, C2007T, C2012T, and C2665T) are not associated with multidrug resistance in Leukemic patients**

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One of the major problems in treating cancer patients is that cancer cells can evolve in drug resistance. Because this form is resistant to multiple anticancer drugs, so called multidrug resistance (MDR), the mode of resistance must be nonspecific, involving drug-efflux transporters. One of the most extensively studied genes involved in MDR is multidrug resistance protein 1(MRP1). We have investigated the possible association between the expression level of MRP1 and occurrence of MDR in 111 patients with acute leukemia (which included 52 patients with acute myelogenous leukemia and 59 patients with acute lymphoblastic leukemia). mRNA level of MRP1 had been determined by quantitative real time RT-PCR and compared to the type of response to chemotherapy. We found that high expression of MRP1 was associated with poor clinical outcome in both AML and ALL patients. In our previous studied we had shown that the increase in MRP1 gene dosage was not responsible for the upregulation of MRP1 expression in leukemic patients.

Therefore, we aimed to investigate the effect of MRP1 polymorphisms on the expression level of it. The T2684C, C2007T, C2012T, and C2665T polymorphisms were genotyped in all the patients and control group. 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.

**P04.098****Study of the effect of MRP1 gene polymorphisms on its mRNA expression in patients with acute leukemic**

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Principle: One of the major problems in treating cancer patients is that cancer cells can evolve in drug resistance. Because this form is resistant to multiple anticancer drugs, so called multidrug resistance (MDR), the mode of resistance must be nonspecific, involving drug-efflux transporters. One of the most extensively studied genes involved in MDR is multidrug resistance protein 1(MRP1).

We aimed to investigate the possible association expression level of MRP1 and occurrence of MDR in leukemic patients wished to test the hypothesis that MRP1 polymorphisms would be predictive of MDR in patients with acute leukemia.

Method: mRNA level of MRP1 was determined in 111 patients with acute leukemia including 52 patients with AML and 59 patients with ALL by RT-PCR and compared to the type of response to chemotherapy.

We found that overexpression of MRP1 is indeed and associated with MDR phenotype in leukemic patients.

Furthermore, the 128G>C, 816G>A, 825T>C, 1299G>C and -260G>C, -275

A>C were genotyped in all the patients and control group.

#### P04.099

#### PHLPP gene is mutated in pediatric acute myeloid leukemia patients and might act as a tumor suppressor gene

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The overactivated PI3K/Akt pathway represents potential therapeutic targets for AML. The protein phosphatase PHLPP has been shown recently to specifically dephosphorylate S473 of Akt which regulates the balance between cell survival and apoptosis. Human *PHLPP* contains an amino-terminal PH domain, a leucine-rich repeat region (LRR), a PP2C-like catalytic core and a PDZ binding motif. So far, there are no described mutations in *PHLPP* gene. In this study, we aimed to understand the architecture of *PHLPP* gene variations in pediatric AML patients. We report here the molecular screening results of 11 exons covering the four domains of *PHLPP* gene in 38 pediatric AML patients. The screening for the presence of a mutation was performed by dHPLC analysis. Mutation detection was accomplished by direct sequencing.

We found the following sequence variations, **exon2** and **3**; 59insA(5.2%), 60C>T(2.8%), 77C>A(2.8%), 109A>T, 289C>A(7.8%), 352C>A(7.8%), 343insA(2.8%), **exon5, 6 and 7**; 599insA(47%), **exon14, 15, 16, 17 and 18**; 1980T>C(5.2%), 1992T>C(5.2%), **exon19**; 3280C>A(13.1%), 3302insA(13.1%), 3303T>C(13.1%), 3407insA(5.2%) and 3611insC(7.8%). The expression analysis of the exons covering the four domains were performed by QRT-PCR. Interestingly, in all patients, we could not detect any expression in LRR domain. We are currently investigating the effect of 599insA (S200R), which might result structural and functional changes at protein level due to the change of the amino acid charges. This is the first study evaluating sequence variations together with the expression of *PHLPP* gene. We propose that *PHLPP* gene might act as a tumor suppressor in AML leukomogenesis and this can provide an important guidepost for the development of diagnostic tools for acute leukemia.

#### P04.100

#### Beta Catenin gene expression and mutation analysis in T-ALL patients and cell line

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The molecular mechanisms regulating the development and differentiation of the haematopoietic system are not clearly understood. Recent studies showed that Wnt signaling proteins play significant roles in both normal lymphocyte development and leukemia pathogenesis. Wnt proteins activate a complex signaling cascade, leading stabilization of  $\beta$ -catenin, which is a key component of the Wnt signaling pathway.  $\beta$ -catenin levels are regulated post-translationally by the canonical Wnt signaling pathway. In this study, we investigate the Wnt/ $\beta$ -catenin pathway activation in T-cell acute lymphoblastic leukaemia (T-ALL) patients and cell lines.

We analyze  $\beta$ -catenin mRNA expression using quantitative real time PCR (QRT-PCR). We measured protein levels by western blot and search for mutations in exon 2-3 of the  $\beta$ -catenin gene in 73 T-ALL patients that were applied to our department for molecular diagnostic purposes and 29 T-ALL cell lines. Wnt signals are transduced by active  $\beta$ -catenin. We showed that T-ALL patients and cell lines have abnormal nuclear accumulation of  $\beta$ -catenin. We also found a  $\beta$ -catenin gene (exon3) mutation in one T-ALL patient. To the best of our knowledge, this is the first study that shows  $\beta$ -catenin gene mutation in T-ALL patients. No mutation was found in any of the cell lines. Our find-

ings reconfirm that Wnt signaling is deregulated in T-ALL by abnormal  $\beta$ -catenin expression. We discuss that  $\beta$ -catenin may play a significant role in promoting leukemic cell proliferation, adhesion, and survival via increased transcription of Wnt target genes.

#### P04.101

#### Deregulation of IRS4 as a result of juxtaposition to T-cell receptor beta in a pediatric t(X;7)(q22;q34)-positive T-cell acute lymphoblastic leukemia

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**OBJECTIVES:** A t(X;7)(q22;q34), an abnormality not previously described in hematologic malignancies, has been molecularly characterized in a pediatric T-cell acute lymphoblastic leukemia (T-ALL). **PATIENTS AND METHODS:** The t(X;7), initially detected by G-banding analysis, was further investigated with fluorescence in situ hybridization (FISH), real-time polymerase chain reaction (PCR) and Western blot analysis. **RESULTS:** FISH disclosed a breakpoint in the T-cell receptor beta locus (7q34) and a breakpoint between RP11-815E21 and RP11-105F23 mapping at Xq22.3. The two genes located closest to this region, i.e. insulin receptor substrate 4 (*IRS4*) and collagen, type IV, alpha 5 (*COL4A5*), were analyzed using real-time PCR. *COL4A5* was not differentially expressed in the t(X;7)-positive sample compared to five T-ALL controls. However, a marked overexpression of *IRS4* was identified in the (X;7)-positive case in relation to the controls. Although the Western blot analysis was suboptimal due to protein degradation, a band representing *IRS4* was found in the t(X;7)-positive T-ALL; this band was not seen in the control samples. **CONCLUSION:** We report the first T-cell neoplasm with a translocation resulting in deregulation of *IRS4*. In fact, this is so far the only reported neoplasia in which a member of the IRS family has been implicated.

#### P04.102

#### Clinical significance and prevalence of T315I mutation in Indian CML patients treated with Imatinib mesylate

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**Background:** The early detection of T315I mutations may allow timely treatment intervention to prevent or overcome resistance.

**Lacunae:** Prevalence of T315I mutation in Indian CML patients.

**Aims:** To detect T315I mutation in CML patients by ASO-PCR. To study prevalence of T315I mutation in Indian CML patients.

**Methodology:** CML patients were diagnosed by RT-PCR.ASO-PCR was done for all 160 patients for BCR-ABL mutations especially for T315I. The patients were evaluated for hematologic and molecular responses, time to progression, survival and toxicity.

**Results :** The study included 160 CML patients. The mutation was detected in 30% of patients (30/160). The median time of Gleevec treatment 25 months.The onset of T315I mutation in 30 patients developed poor prognostic factors in these patients. 25/30 lost hematological & molecular responses.20/25 progressed to advanced stage. T315I mutations had proven to be fatal & is soul cause of Imatinib resistance in our patients. Survival and time-to-progression curves were obtained from Kaplan-Meier method.

**Discussion :** India is a developing country, the patients cannot afford expensive tests like sequencing for T315I mutation. So we had standardized ASO-PCR for routine screening of T315I mutations in our patients.

**Conclusion:** ASO-PCR proved to be very economical, sensitive and rapid technique for detection of known T315I mutations and is even sensitive than mutation detection by sequencing. The early detection by ASO-PCR assay proved to be helpful in clinical management of therapeutic decisions in CML patients.

**P04.103****Integrated CGH and MicroRNA analysis in Mantle Cell Lymphoma reveals miRNA signatures associated with specific cytogenetic changes**

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Mantle Cell Lymphoma (MCL) is characterized by a t(11;14) translocation, which leads to Cyclin D1 overexpression. Although CGH and Expression profiling data in MCL has been investigated, until now no equivalent studies have been performed for miRNA. Here we explored the relation between miRNA expression profiling and CGH microarray data from a series of 20 MCL cases and 5 reactive tonsils

MCL presents a unique miRNA signature revealing overexpression of 15 miRNAs and downregulation of 50 miRNAs in at least 50% of the cases, when compared with the average expression level of the controls. Some of these miRNAs have already been described in other tumor types, such as miR-143 and miR-145 downregulated in B-cell malignancies.

CGH analysis in this series identifies a series of changes, roughly coinciding with those described. We have investigated whether chromosomal gains and losses could explain the miRNA MCL signature.

A microRNA profile has been identified as associated to the most frequently chromosomal aberration described in MCL. Thus losses of 9p21-pter, 1p, 13q, 11q21; and gains of 15q and 3q were associated with distinctive miRNA changes.

Taken together, MCL seems to combine a disease-specific miRNA signature that correlates with specific chromosomal changes.

**P04.104****A rare karyotype including t(2;17) in a patient with myelodysplastic syndrome-derived acute myeloblastic leukemia**

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Myelodysplastic syndrome (MDS) is a clonal disorder characterized by dyshematopoiesis and high susceptibility to acute myeloid leukemia (AML). Complex chromosomal aberrations are present in ≤30% of patients with primary myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) and are associated with a poor prognosis. We report a 45 year-old patient with MDS-AML. The Wright-Giemsa stained peripheral blood smears showed blast cells (WBC: 3180/mm<sup>3</sup>, Hb: 9.69g/dl, Plt: 21100/mm<sup>3</sup>). An abnormal karyotype 44,XX, t(2;17),-16,-18 was obtained on G-banded metaphases from unstimulated bone marrow aspirate cell culture. Cytogenetic studies of AML showed that isolated -16 and -18 were significantly more common. To our knowledge, isolated t(2;17) is not described in AML to date. This is the first report of a patient presenting with a AML with t(2;17),-16,-18. We evaluated the outcome of the treatment and the prognosis of the patient. As in our patient, this rare karyotype may be associated with poor prognosis.

**P04.105****Increased incidence of monoclonal B-cell infiltrate in chronic Myeloproliferative disorders. Report of four cases**

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**INTRODUCTION:** The coexistence of chronic myeloproliferative disorder(CMPD)and B-cell chronic lymphocytic leukemia(CLL)in the same patient is rare.

**AIM OF THE STUDY:** We report 4 cases of CMPD/CLL simultaneously occurred.

**PATIENTS**

1<sup>st</sup> patient:An Albanian man 57y with polycythemia Vera(PV).

2<sup>nd</sup> patient:A woman 73y with essential thrombocythosis (ET)

3<sup>rd</sup> patient:A man 83y with ET

4<sup>th</sup> patient:A man 78y with ET

In paraffin sections of the bone marrow samples was found 15-18% of monoclonal lymphocytic infiltrate CD5+,CD23+,CD10-,CD3-,ZAP-70-,CD79+simultaneously with the findings of CMPD.

The patients were untreated and the trephine biopsies derived from the primary diagnostic procedure.In the Albanian patient a cervical lymph node(LN)was biopsied and the cell population was of Borigin

**METHODS:** Bone marrow specimens(BM)and PB lymphocytes were cultured using standard techniques.Thirty GTG banded metaphases were analyzed (ISCN2005).

**RESULTS:** The karyotypes looked normal.For FISH we used the LSI IGH dual color, break apart rearrangement probe, LSI BCR/ABL ES dual color translocation probe and WCP CEP-8(VYSIS).

Two hundred interphase nuclei were counted.Up to25% of the cells carried the BCR/ABL translocation, and the IGH rearrangement.

There was also monosomy of #8 in more than 10% of the cells

**CONCLUSIONS:** Although the coexistence of CMPDand CLL is rare,we established that these two conditions in the PB,in the BM and in one LN is more frequent than expected.That implies that there is a predisposition for the development of monoclonal B-cell population. With FISH technique we demonstrated the coexistence of two diseases.Since we studied simultaneously PB,BM and a LN,we believe that CMPD and CLLsupport the hypothesis of multi step cancerogenesis

**P04.106****Molecular cytogenetic study of 69 patients with myelodysplastic syndromes and complex chromosomal aberrations**

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Complex chromosomal aberrations (CCA) are detected in 10-20% patients with myelodysplastic syndromes (MDS), and are associated with drug resistance and poor outcome. Precise identifications of chromosomal regions involved in CCA could help in detection of cryptic, recurrent, prognostically significant aberrations and in identification of candidate genes involved in leukemogenesis.

During the last 6 years 590 patients with MDS were examined at diagnosis and in 69 of them CCA were ascertained. Chromosomal aberrations were verified by FISH with locus specific probes (Abbott-Vysis, Des Plaines, Illinois, USA), and by mFISH/mBAND with the "XCye" probe kits (MetaSystems, Altluessheim, Germany).

The most frequently involved in complex rearrangements was chromosome 5 (58x) followed by chromosomes 3 (27x), 17 (25x), 12 (23x), 11 (20x) and 7 (20x). Parts of deleted chromosome 5 were in many cases translocated into other chromosomes. The most recurrent partners of chromosome 5 in translocation were chromosomes 17 (6x), 12 (6x), 3 (3x) and 7 (3x). Pure monosomy of chromosome 5 was proved in one case only thus showing that monosomy 5 in this cohort was not an isolated entity. The most frequent breakpoints were 5q31 (25x), 5q13.3 (24x), 5q12 (5x) and 5q14 (5x). Presence of CCA at diagnosis was connected with poor response to therapy and short survival (mean 5 months).

Finding of new chromosomal rearrangements and breakpoints might lead to discovery of genes, involved not only at the origin but also into pathways leading to progress of malignancy.

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**P04.107****Trisomy 9: A rare chromosomal abnormality in MDS**

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Myelodysplastic Syndrome (MDS) is a clonal disorder of haematopoietic stem cells and result in progressive cytopenia and defects in erythroid, myeloid and megakaryocytic maturation. Clonal chromosomal abnormalities have been reported in 30 to 60% cases of MDS. As a result conventional cytogenetics plays a prominent and well established role in determining the contemporary diagnosis, prognosis and grading of this disorder. The chromosomal abnormalities are predominantly characterized by partial/ total chromosomal loses or chromosomal gains. The chromosomal abnormalities include mainly -5/del (5q), -7/del (7q), del (11q), del (12p)/ (12q), -y and +8. In an attempt to assess the frequency and type of cytogenetic abnormalities, cytogenetic analyses of 20 cases of MDS was done. Chromosomes

preparations were obtained by GTG banding of blood and bone marrow cells. On presentation 30% of cases revealed 46, XX and 46, XY normal karyotypes and 70% of cases revealed abnormal karyotypes del(5q), -7, del(6q), del(11q). Out of 20 cases 1 case revealed an addition of chromosome 9, 47,XX+9(90%) and 46,XX(10%) metaphase. Trisomy 9 is a rare chromosomal aberration found in MDS, to the best of our knowledge this is the first published report of trisomy 9 as a sole chromosomal aberration in MDS. The presence of trisomy 9 in such cases carries a poor prognosis and thus cytogenetic studies help to correlate the genotype with the phenotype.

#### P04.108

#### Complex cytogenetic findings in bone marrow of an elderly patient with chronic idiopathic myelofibrosis

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Chronic idiopathic myelofibrosis is a chronic myeloproliferative disorder characterized by splenomegaly, myeloid metaplasia and reactive bone marrow fibrosis. Clonal cytogenetic abnormalities are observed in 30 to 75% of the patients. Among these, trisomy 1q, 20q-, 13q- and +8 are the most common aberrations. Here we report a 70-year-old male patient with massive splenomegaly. His leukocyte count was 2300/mm<sup>3</sup>, hemoglobin 6,5 mg/dl and thrombocyte count 700 000/mm<sup>3</sup> on admission. Bone marrow biopsy revealed signs of chronic myeloproliferative changes and dysmegakaryopoiesis and no specific diagnosis was established. During disease classification studies, he received hydroxyurea treatment, splenic radiotherapy and multiple blood transfusions. The clinical course worsened in the following months and the second bone marrow biopsy revealed myelofibrosis. Cytogenetic analysis of the bone marrow sample revealed a karyotype reported as 46,XY,del(9)(q22q34),t(8;17;21)(q22;q21;q22)[23]/46,XY[2], with a previously undefined three-way translocation and a deletion in chromosome 9. The patient died shortly thereafter. Karyotype analysis of the bone marrow is an integral part of diagnosis in myeloproliferative disorders, especially as a discriminative tool in ruling out reactive conditions.

#### P04.109

#### Inducible expression of the oncogenic transcription factor EVI1 in human myeloid cells leads to phenotypes characteristic of myelodysplastic syndromes (MDS)

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The *EVI1* gene, which codes for a zinc finger transcription factor, is overexpressed in subsets of patients with acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and myelodysplastic syndromes (MDS). Its overexpression in AML has been studied intensively because it is associated with particularly aggressive disease. However, recent bone marrow transduction/transplantation studies in mice have shown that *EVI1* overexpression by itself causes MDS, while AML arises only in the presence of additional, cooperating genetic events.

To gain a better understanding of the biological properties of *EVI1*, an HA-tagged version of the human *EVI1* cDNA was expressed in human U937 myelomonocytic cells in a tetracycline regulable manner. Induction of *EVI1* in this system strongly inhibited cellular multiplication, an effect that was in part due to cell cycle arrest, and in part to increased rates of apoptosis. Exposure of *EVI1* expressing cells to differentiation stimuli caused cells to die rather than to differentiate. The *c-myc* gene, which is implicated in the control of cellular proliferation, was downregulated rapidly after the induction of *EVI1*, suggesting that it may be a direct target of this transcription factor, and may play a role in its phenotypic effects.

The phenotypes observed after induction of *EVI1* correspond well to what would be expected for a gene involved in the pathogenesis of MDS, and have also been observed with other MDS-associated oncogenes. We have therefore established a suitable model system to explore the role of *EVI1* in the pathogenesis of this fatal disease.

#### P04.110

#### Familial myelodysplastic syndromes

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The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders of hematopoietic stem cells, whose manifestation is cytopenia, hypercellular and dysplastic bone marrow, often with increased amount of blasts. The pathogenesis of the majority of MDS remains unexplained. It is regarded that genetic predisposition and exposure to toxic environmental agents contribute to genetic mutations in MDS.

We report an adult MDS family with 6 siblings, of which two presented myelodysplastic syndrome (MDS), namely refractory cytopenia with multilineage dysplasia (RCMD), at the ages of 37 and 49, respectively. Conventional cytogenetics showed complex karyotypes, at diagnoses, in both:

44,XX,del(5)(q13q33),-7,-9,der(15;21)(q10;q10),-21,+mar[9]/46,XX[10] in the propositus, and 46,XY,-3,del(5)(q13q35),+8,der(12)t(3;12)(q13;p13) [20] in the second one. The propositus developed acute leukaemia and underwent an allogenic transplantation of peripheral blood progenitor cells. She relapsed and eventually died of sepsis eight months post-transplantation. In order to know the status of the 4 other siblings, we performed morphological and cytogenetics bone marrow analyses. Since del(5)(q31q33) was the only chromosomal abnormality common to both complex karyotypes, we decided to investigate all the samples by FISH with the specific probe for 5q31. Surprisingly, we found that in the propositus the deleted chromosome was an i(5)(p10;p10), and one of the healthy brothers showed 12% of deletion 5q.

We postulate that in this family an inherited mutator effect is present and that it causes a karyotype instability, which leads to MDS/AML, with an unstable 5 chromosome.

#### P04.111

#### HFE C282Y mutation as a genetic modifier influencing disease susceptibility for chronic myeloproliferative disease

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The Janus kinase 2 (JAK2) V617F point mutation is a common clonal alteration in chronic myeloproliferative disorders (CMPD). Genetic variations influencing susceptibility of CMPD have not been recognized previously. The aim of our study was (i) to establish V617F mutation status in CMPD patients (ii) to confirm associations with distinct clinical characteristics, and (iii) to examine the potential associations of CMPD development with genetic modifiers of iron metabolism (HFE C282Y, H63D and TFR S142G). HFE C282Y was genotyped in 328 CMPD-patients and 996 blood donors, HFE H63D and TFR S142G in CMPD patients and 171 first time blood donors. JAK2 V617F mutation was tested in CMPD patients and 122 repeated blood donors. The frequency of JAK2 V617F was 75.9% (249/328) in the CMPD group. At presentation, significantly elevated hemoglobin levels were found in V617F-positive patients compared to V617F-negative counterparts ( $p<0.000$ ). Vascular complications were more common in V617F-positive patients ( $p=0.039$ , 26.6% vs. 15.2%). Decreased HFE C282Y allele frequency (AF+-95%CI) was found in the CMPD-group (1.8+-1.0%) compared to controls (3.4+-0.8%;  $p=0.048$ ). TFR S142G AF was significantly reduced among V617F-negative CMPD-patients (34.8+-7.6%) compared to controls (47.8+-5.4%,  $p=0.02$ ). The age of CMPD onset and the rate of different complications were not different according to HFE or TFR genotypes. We found that HFE C282Y variant may be associated with a protective role against CMPD especially among V617F-positive cases. Since chronic iron deficiency or latent anemia may trigger disease susceptibility for CMPD, HFE C282Y positivity may be a genetic factor influencing this effect.

**P04.112*****NBS1 657del5 mutation in patients with myelodysplastic syndromes (MDS)***

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Myelodysplastic syndromes (MDS) are the most common group of hematological disorders in persons older than 60-year age. MDS are characterized by elevated apoptosis in bone marrow and peripheral blood cytopenias. Since more than one third of all MDS patients develop acute leukemia MDS are considered preleukemic states.

*NBS1* gene encodes for the nibrin (p95 protein). p95 acts in a double-strand DNA break repair as the part of MRE11/RAD50 double-strand break (DSB) repair complex. Nibrin is involved in cell cycle checkpoint, meiotic recombinations and telomere maintenance.

*NBS1* gene mutations are recognized as a main molecular event in development of Nijmegen breakage syndrome (NBS). NBS is chromosome instability syndrome resulting in microcephaly, growth retardation, immunodeficiency and predisposition to different types of cancer. NBS patients have a particularly high predisposition to lymphoid malignancy.

Independently from Nijmegen breakage syndrome, alterations of *NBS1* gene sequence (mostly nucleotide substitutions and deletions) and expression are found in number of malignancies. Deletion of 5bp in exon 7(657del5) is the most often *NBS1* gene mutation found in Slavic population (so-called Slavic mutation).

In this study we have analyzed *NBS1* 657del5 mutation in a cohort of 71 MDS patients. Patients DNA were obtained from bone marrow microscope slides splices. To detect mutation we have compared PCR products of MDS patients DNA with control DNA containing *NBS1* 657del5 mutation. Only one patient harbored mutation. According to this study, *NBS1* 657del5 mutation may not be important event in evolution of MDS.

**P04.113*****A pediatric MDS patient with 5q31 deletion***

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Myelodysplastic syndrome (MDS) includes the ineffective proliferation or production of cells in bone marrow (BM) leading to peripheral blood cytopenia and a preleukemic state. The pathogenesis of MDS involve a multistep process involving two or more genetic alterations that lead to alteration in cellular function that cause clonal proliferation of an abnormal stem cell. In the present study, we report on a childhood patient with MDS. The patient had 5q31 deletion by conventional cytogenetics and fluorescence in situ hybridization (FISH) analysis with a specific FISH probe at the diagnosis. We present the clinical features, genetics and immunologic analysis at the diagnosis and also followed-up results.

**P04.114*****FISH studies in multiple myeloma patients***

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Multiple Myeloma (MM) is a heterogeneous disease regarding its clinical and genetic properties. Cytogenetic studies are valuable diagnostic tools in MM patients both at the time of diagnosis and during clinical follow up. Although conventional karyotyping is the first choice, fluorescent in situ hybridization (FISH) is also important in detecting abnormalities that cannot be detected by karyotype analysis. Conventional cytogenetic analysis and FISH results of bone marrow samples of 36 MM patients at the time of diagnosis have been evaluated in the current study. Three probes for chromosome 13q (RB1, D13S319, D13S25), one for 14q32 (IgH) and one for 17p13 (p53) have been used for hybridization with fixed cells. Conventional cytogenetic results revealed that 20 patients (55.5%) had normal karyotypes, whereas 8

(22.2%) had numerical or structural chromosomal abnormalities. We did not find appropriate metaphases for chromosome analysis in 8 (22.2%) patients. FISH analyses revealed at least one or more abnormal results in 25 (69.5%) cases, whereas 11(30.5%) cases had no abnormal findings. 14q32 rearrangement was the most common finding in FISH analyses and has been detected in 21 cases (58.3%). 13q deletion and 17p deletion have been detected in 11 (30.5%) and 5 (13.9%) cases, respectively. In evaluating MM patients, FISH studies including 14q32 and 17p13 chromosome regions may yield quite significant results during clinical follow up of the disease which has a multistep pathogenesis.

**P04.115*****Cytogenetic studies among patients with myelodysplastic/myeloproliferative diseases***

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The myelodysplastic/myeloproliferative diseases (MDS/MPD) are clonal myeloid disorders that possess both dyspastic and proliferative features. Clinical symptoms are caused by complications resulting from cytopenia, dysplastic cells with abnormal function, leukemic infiltration of various organ systems, fever and malaise.

We are reporting the chromosome studies among 34 patients which referred to us with diagnosis of MDS/MPD by Hematologists/Oncologists during the last 12 months. Chromosome preparations were obtained from lymphocyte cultures and analyzed after GTG-banding and HR-banding.

30 patients (88.24%) showed normal karyotype. 4 patients (11.76%) had chromosome abnormalities.

Only one 67-year-old man patient who were diagnosed by MDS, showed abnormal karyotype of : mos 46, XY, add (20)(q) / 46, XY.

Three patients with diagnosis of MPD also showed chromosome aberrations as follows : First patient was a 32-year-old woman and her karyotype was : mos 46, XX, t (2, 11) (q33, q23) / 46, XX, del 2 q33 +7+9 -11+18 -X / 46,XX. Second one was a 56-year-old man with karyotype:

mos 47, XY, +22 / 45, XY, -7 / 46, XY.

Third case was a 57-year-old man and had a karyotype of: mos 92, XXYY / 46, XY.

All the patients did not have a Philadelphia chromosome.

**P04.116*****Cytogenetic study of 25 myelodysplastic syndrome***

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The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal hematopoietic stem cell disorders. Cytogenetic analyses in bone marrow samples of MDS patients have both pathophysiologic significance and prognostic implications. Chromosomal abnormalities are found in 40 - 70 % of the patients with MDS, and the characteristic chromosomal abnormalities are del(5q), -5, del(7q), -7, +8, del(11q), del(12p), del(13q), del(17p), del(20q), +21.

We analyzed cytogenetically twenty five samples of MDS patients. There were 12 female and 13 male patients with an age-range of 51 to 87 (median 69). Cytogenetic abnormalities were observed in 40% of patients. The most common abnormalities were monosomy of chromosome 7 and trisomy of chromosome 8. Complex karyotypes were found in 3% of the cases.

Despite the small number of cases studied the results obtained corroborate those described in the literature and the importance of the cytogenetic study in the prognosis of MDS.

**P04.117*****Role of JAK2 V617F mutation in myeloproliferative disorders***

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Chronic Myeloproliferative disorders (MPD) polycythaemia vera(PV), essential thrombocythaemia(ET), and idiopathic myelofibrosis(IMF) form a range of clonal haematological malignant diseases characterized by proliferation of one or more lineages of the myelo-erythroid se-

ries. The identification of the JAK2-V617F mutation is an exciting new discovery in the field of MPDs. This acquired mutation is characterized by G→T nucleotide substitution at 1849 position leading to valine to phenylalanine substitution at amino acid position 617 (V617F) of the JAK2 tyrosine kinase protein which results in constitutive JAK2 activation that promotes proliferation. **Objective:-** To study the role and frequency of the JAK2 mutation in myeloproliferative disorder patients.

**Materials &Methods:-** 60 MPD patients(30PV,16 IMF,14ET diagnosed by bone marrow biopsy), 90 AML and 70 age& sex matched controls were studied .DNA was extracted from peripheral blood mononuclear cells and Allele Specific PCR was performed for V617F mutation.

**Results:-** Majority of PV patients (28/30;93.4%) had V617F mutation whereas the frequency of the mutation was lower in IMF and ET patients(7/16; 43.7% and 7/14;50% respectively).The mutated patients had significantly higher mean haemoglobin and platelet count compared to JAK2 wild type patients and controls.(p=0.01). Higher hematocrit and splenomegaly was observed in V617F patients. Only one AML patient and none of the 70 controls had VF617 mutation . **Conclusion:-** Thus presence of the mutation confers a proliferative and survival advantage. Identification of the Val617Phe JAK2 mutation lays the foundation for new approaches to the diagnosis specially PV and classification. JAK2 can be new gene for the targeted therapy in myeloproliferative disorders.

#### P04.118

#### Importance of using JAK2 mutation in the diagnosis of myeloproliferative diseases

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Chronic myeloproliferative disorders (CMPDs) are a group of various clonal haematological malignant diseases including chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (CIMF). Except for CML with a genetic abnormality the t(9;22), the molecular mechanisms underlying the CMPDs have not been identified. Recently, several research groups shown that a significant proportion of patients with non-CML CMPDs have a missense somatic mutation in JAK2 gene that substitutes phenylalanine for valine at residue 617 (JAK2 V617F). JAK2 is a nonreceptor tyrosine kinase (TK). Pseudokinase domain of JAK2 in haematopoietic cells is responsible for the constitutive activation of molecular signalling pathway and takes control of cell proliferation in CMPDs.

DNA from 306 samples was isolated from peripheral blood granulocytes in patients with CMPDs and genotyped for the JAK2 V617F (G>T) mutation. To identify the mutation we used a real-time polymerase chain reaction assay using fluorescent hybridization probes and melting curve analysis. In a few cases that are JAK2 V617F negative and have the non-CML CMPDs phenotype we detected novel mutations in JAK2 exon 12 by direct sequencing. The detection of the JAK2 mutations could be useful in the diagnostics of myeloproliferative disease patients.

#### P04.119

#### Association between antral nodularity, severe gastritis, positive serology, age, gender, ABO blood group and Helicobacter pylori in children

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Hp causes one of the most widespread infections worldwide. It is well known that blood group antigens are related to the development of peptic ulcer and gastric carcinoma.

Association between virulence factors of Hp, severe gastritis, age, gender and ABO blood group.

25 patients underwent to esophagogastroduodenoscopy with antral biopsy for a suspicious upper gastrointestinal disease. In all of them serum sample were assayed for IgG antibodies to CagA and ABO blood groups were determined.

19 children (76%) were Hp positive by histopathology and urease rapid test. 15 of these 19 (79%) Hp positive patients were positive for CagA serology. At endoscopic examination of the 19 infected children, hyperemia of the gastric antrum was observed in 7 (37%) patients and antral

nodularity in 12 (63%) children. The histologic examination of all infected patients showed an active and chronic gastritis. The children CagA positive presented more intense hyperemia of gastric antrum, important lymphoplasmacellular infiltrate and a degenerative and vacuolar lesions of gastric epithelium. The 6 (24%) non infected patients, also negative for CagA serology, had a normal gastric finding. As expected from previous studies, we found that seropositivity for Hp increased with age and the rate of Hp infection was not significantly different in boys and girls. Similarly, Hp serological status was not significantly different between subjects of different ABO blood groups. Despite epidemiological evidence of increased peptic ulcer disease in ABO blood group O subjects, we found no association between Hp infection and ABO blood groups.

#### P04.120

#### Low Expression of ARHI Contributes to Glial Tumor Development

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Although the ARHI gene shows 60% sequence homology to the Ras proto-oncogene, it is the first maternally imprinted tumor suppressor gene identified in the Ras family. ARHI gene is constitutively expressed from the paternal allele of normal breast, ovarian, heart, liver, pancreas, thyroid, brain tissues and its expression is lost or markedly downregulated primarily in breast, ovarian, pancreas and thyroid tumor tissues. In this study, we investigated the mRNA expression, LOH (loss of heterozygosity) and methylation analysis of ARHI gene in tumor and peripheral blood samples of 21 cases with glial tumor, and 7 normal brain tissue samples. Evaluation of the ARHI gene expression levels by RT-PCR revealed reduction in 7 of 21 glial tumor samples (33.3%). Analysis of LOH was performed by fragment analysis using 5 labeled polymorphic markers specific for 1p31 region. LOH was detected in 2 of 21 cases (9.5%). Methylation status of CpG island II of 5 cases was evaluated using COBRA (combined bisulfite restriction analysis) and RFLP. Results indicated complete lack of hypermethylation in the CpG island region II. Our results suggest that silencing of ARHI tumor suppressor gene may play a role in the glial brain tumor development.

#### P04.121

#### Analysis of ATM case-control mutation screening data

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The susceptibility gene for Ataxia telangiectasia, ATM, recently has been established as an intermediate-risk breast cancer (BC) susceptibility gene. However, the answer to the question "what sort of sequence variation in ATM confers increased risk of BC" has been controversial. To address this question, we have pooled available ATM mutation screening data and carried out a combined analysis of truncating variants, splice junction variants, and rare missense substitutions (carrier freq ≥ 1%). The analysis of rare missense substitutions was accomplished by constructing a sufficiently informative protein multiple sequence alignment of ATM from full-length sequences of seven species and using a missense analysis program Align-GVGD with developed new classifiers. Systematic case-control analysis of the missense substitutions independently provided evidence that ATM is a BC susceptibility gene. We found that a combined analysis of truncating mutations, splice junction mutations, and rare missense substitutions provides stronger evidence that ATM is a BC susceptibility gene than simple consideration of truncating plus splice junction variants alone. We also found significant evidence of risk both in truncating plus splice junction variants and in the in silico predicted highest-risk class of missense substitutions. Taken together, these results led us to two conclusions: (1) Careful analysis of missense substitutions by measuring risk attributable to rare missense substitutions in a known or candidate suscep-

tibility gene will have real utility in case-control mutation screening. (2) The attributable fraction of rare missense substitutions in ATM for risk of BC is approximately equivalent to that of truncating and splice junction variants.

#### P04.122

#### Alterations in genes encoding sarcoplasmic-endoplasmic reticulum Ca<sup>2+</sup> pumps in association with head and neck squamous cell carcinoma

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Recent studies have suggested that perturbation of intracellular Ca<sup>2+</sup> homeostasis or signalling could contribute to cancer development. The purpose of this study was to evaluate whether germline variants of the ATP2A2 and ATP2A3 genes might act as susceptibility alleles in head and neck squamous cell carcinoma. In both genes, we identified eight different alterations in 11 patients with head and neck squamous cell carcinoma (11/79;  $P = 0.0002$ , odds ratio = 0.054, 95% confidence interval = 0.0069-0.4236). We also detected low expression level of both genes in connection with some of alterations, but could not correlate low expression level with methylation in the promoter region of either gene. The results suggest that Ca<sup>2+</sup> pumps of sarcoplasmic-endoplasmic reticulum are involved in an increased susceptibility to develop head and neck squamous cell carcinoma in humans.

#### P04.123

#### Neuroendocrine carcinoma in Birt-Hogg-Dubé syndrome - do FLCN mutations contribute to malignancy?

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Birt-Hogg-Dubé syndrome (BHD) is a dominantly inherited disorder characterized by an increased risk of developing kidney cancer, pneumothorax as a consequence of lung cysts and benign hair follicle tumors called fibrofolliculomas. It is caused by mutations in the gene coding for folliculin, a protein involved in mTOR signaling. As the relative risk of developing renal malignancies is around 5, BHD syndrome is generally considered to be a relatively benign condition for which annual follow-up suffices. However, the possibility that the pre-existing gene defect might act to modify the behavior of other cancers that arise in patients with BHD syndrome has so far not been considered. We describe a patient with BHD syndrome who succumbed to an extremely malignant neuro-endocrine tumor of prostatic or bladder origin within 6 weeks of its discovery. Tumor tissue showed elevated staining of phosphorylated mTOR and phosphorylated p70S6K, as did foci in clinically normal skin and kidney, confirming that folliculin is a negative regulator of mTOR signaling. We propose that the behavior of this cancer might have been modulated by the germline FLCN mutation and propose that patients with BHD syndrome be more closely monitored than is currently the case.

#### P04.124

#### Gene expression in superficial transitional cell carcinoma using oligonucleotide microarrays

J. Mares<sup>1</sup>, M. Szakacsova<sup>2</sup>, F. Zelezny<sup>3</sup>, J. Klema<sup>3</sup>, V. Soukup<sup>2</sup>, J. Dusko<sup>4</sup>, A. Sartori<sup>5</sup>, M. Babjuk<sup>2</sup>;

<sup>1</sup>*Inst. Biol. and Med. Genet. 2nd Med. Faculty, Prague, Czech Republic,* <sup>2</sup>*Clin. of Urol., 1st Med. Faculty, Prague, Czech Republic,* <sup>3</sup>*Dept. Cybernet, Czech Tech. University, Prague, Czech Republic,* <sup>4</sup>*Inst. of Pathol. Anatomy, 1st Med. Faculty, Prague, Czech Republic,* <sup>5</sup>*Applied Biosystems, Heidelberg, Germany.* The prediction of tumour recurrence in patients with superficial bladder tumours presents several challenges in clinical management. For the improvement of recurrence prognosis in these patients we investigated gene expression and identified differences between superficial bladder tumours without recurrence during period of two years (10 patients) and with early recurrence (12 patients), which might explain differences in the biology and clinical outcomes High-density oligonucleotide microarrays (29,019 genes, AB) were used to analyze the transcript profiles of 22 superficial bladder tumours: 19 pTa and 3 pT1, grading 5 G1 and 17 G2. Statistical analyses were applied to investigate the ability of the genes to identify patients without recurrence during period of two years and with early recurrence. Initial screening using the GeneSpring and Bioconductor software tools revealed a putative set

of about 120 genes associating with the recurrence class. Significant differences were observed by HOXA10, GPNMB, TCN1, INA, H19, AURKC, FABP3 and PLOD2 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 is supported by MSM 0021620808 and IGA NR 8934-3.

#### P04.125

#### Gene expression in superficial transitional cell carcinoma using oligonucleotide microarrays

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The prediction of tumour recurrence in patients with superficial bladder tumours presents several challenges in clinical treatment. For the improvement of recurrence prognosis in these patients we investigated gene expression and identified differences between superficial bladder tumours without recurrence during period of two years (10 patients) and with early recurrence (12 patients), which might explain differences in the biology and clinical outcomes High-density oligonucleotide microarrays (29,019 genes, AB) were used to analyze the transcript profiles of 22 superficial bladder tumours: 19 pTa and 3 pT1, grading 5 G1 and 17 G2. Statistical analyses were applied to investigate the ability of the genes to identify patients without recurrence during period of two years and with early recurrence. Initial screening using the GeneSpring and Bioconductor software tools revealed a putative set of about 120 genes associating with the recurrence class. Significant differences were observed by HOXA10, GPNMB, TCN1, INA, H19, AURKC, FABP3 and PLOD2 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 is supported by MSM 0021620808 and IGA NR 8934-3.

#### P04.126

#### The Association of CYP1A2, CYP2D6, GSTM1, GSTP1 and GSTT1 Gene Polymorphisms with Bladder Cancer

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Bladder cancer is the fourth most common cancer in men and the eighth in women in the western world. The aim of this study was to investigate the relationship between bladder tumor and variants of the cytochrome p450 (CYP), family 1, subfamily A, polypeptide 2 (CYP1A2) C734A, CYP2D6 G1934A, the glutathione S-transferase (GST), family M, subfamily 1 (GSTM1 null), GSTT1 null and GSTP1 I105V which play important roles in xenobiotic metabolism. In this study, we investigated the distribution of these polymorphisms in 135 bladder cancer patients and 128 age-matched healthy individuals as controls. The polymorphisms were analyzed using polymerase chain reaction (PCR) - restriction fragment length polymorphism (RFLP) assay and multiplex PCR method. Genotype and allele frequencies were calculated, and their associations with bladder cancer risk are calculated, and their association with bladder cancer risk or demographic factors, smoking status, and tumor stage was investigated. The prevalence of GSTT1 null genotype in cases was 23%, compared with 7% in the control group (OR, 0.254, %95 CI, 0.115-0.558,  $p=0.001$ ). No association was observed between CYP1A2, CYP2D6, GSTM1, and GSTP1 genes polymorphisms and bladder cancer. There was association between smoking status and bladder cancer (OR, 1.914, %95 CI, 1.055-3.472,  $p=0.033$ ), but there was no statistically significant association between demographic factors, tumor stage, tumor grade and bladder cancer. These data seem to indicate that GSTT1 gene polymorphism may be associated with bladder cancer in this Turkish population.

**P04.127****FGFR3 mutations and 3p, 9p, 9q & p53 deletions in noninvasive bladder cancer**

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The bladder cancer is one of the most severe oncological diseases. Importance of the development of new diagnostic clinical markers is dictated by its high incidence and aggressive tumor growth. Cancer development is a complex, multistage process involving various genetic and epigenetic alterations. In our study we have tried to establish associations between several genetics alterations (loss of heterozygosity (LOH) at 3p, 9p, 9q and p53 loci and S249C activating mutation in *FGFR3*) and tumor clinical phenotype (tumor differentiation, type of growth and a non-recurrent period). During the last year and the half we have studied 40 matched samples (blood and tissue) from patients with primary bladder cancer and 35 samples from patients with recurrent bladder cancer, of which 17 patients demonstrated recurrence within one year. All samples were divided into groups by differentiation rate: 53 samples classified as G1+G2 and 22 as G3. By growth type 53 samples were unifocal and 39 multifocal. LOH at 3p, 9p, 9q and p53 loci were detected by microsatellite analysis, and SSCP and direct sequencing sought for identification of *FGFR3* activating mutations. Statistical analysis of the results included comparison of the patients' clinical groups by Fisher's exact test, calculation of odds ratios and corresponding 95% confidence intervals, with GraphPad InStat v.3.5 software. Our results demonstrate that 9p deletions are significantly more frequent in tumors with high recurrence rate (within one year). *FGFR3* mutations are prevalent in G1+G1 group of tumors. No significant differences were found between tumors with various growth types.

**P04.128****Evidences for cancer cells showing stem features from human bladder Transitional Cell Carcinoma**

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Transitional cell carcinoma (TCC) is the most common type of bladder cancer accounting for more than 90-95% and it often recur (75% after 5 years).

Emerging evidence has suggested that the capability of a tumor to grow, propagate and recur is dependent on a small subset of cells within it, termed cancer stem cells (CSCs). Current failure of cancer therapies may be due to their lower effect on CSCs that are resistant and retain their full capacity to divide and restore the tumor cell mass. Although data have been provided to support this theory in human blood, brain, and breast cancers, the identity of bladder cancer stem cells has not been determined. Here, we report the initial findings towards the isolation and preliminary characterization of a putative CSCs population from human bladder TCCs. These cells, isolated from fresh surgical specimens, were induced to proliferate in vitro in serum-free medium containing the mitogenic growth factors EGF and bFGF. The proliferating cells generated within 48 h detached spheres that showed clonal origin. Cells also resulted positive by immunofluorescence for the stem cells' markers CD133, Oct-4, Nestin and Cytokeratins. We also conducted a cytogenetic study on fresh chromosome spreads and, when possible, on chromosome spreads after different time of culture and a parallel molecular cytogenetic study by FISH on paraffin embedded tissue sections and on fresh and after culture nuclei. We found important karyotype changes by culture selection, losing the complexity present in fresh tumors and a marked molecular heterogeneity between the tumors.

**P04.129****Reliable Methylation Analysis for Epigenetic Research - Novel Technologies offering a complete and standardized workflow**

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QIAGEN GmbH, Hilden, Germany.

The analysis of changes in DNA methylation is challenging due to the lack of standardized methods for providing reproducible data. Here we present a complete and standardized workflow for methylation analysis.

**Bisulfite Conversion:** QIAGEN's EpiTect Bisulfite technology represents a unique system for DNA protection against DNA degradation which guarantees the highest conversion efficiency of ≥ 99% and highest DNA quality.

**Whole bisulfiteome amplification:** Since the quantity of converted DNA is often limited, we here present a novel technology for the reliable and representative amplification of the entire bisulfite converted genomic DNA \_ the bisulfiteome \_ to overcome limitations in methylation analysis derived by limited DNA amounts.

**Dedicated PCR technology for methylation analysis:** Methylation Specific PCR (MSP) reactions often require extensive optimization. We present a mutant Taq DNA Polymerase that has been genetically engineered to increase primer extension specificity through better discrimination of 3' single base mismatches of the primer.

For highly sensitive TaqMan probe based real-time PCR, we have developed a novel reagent that yields accurate methylation analysis in real-time. A selection of pre-developed MethylLight assays for a first set of genes will also be made available.

**Assay control reagents:** Assay design and the success of methylation analysis by PCR can be facilitated and assessed by the use of standardized human control DNAs.

With its newly introduced EpiTect solutions, QIAGEN makes available standardized, pre-analytical and analytical solutions from DNA sample collection, stabilization and purification, to bisulfite conversion and real-time or endpoint PCR methylation analysis or sequencing.

**P04.130****Karyotype alterations of CD133(+) stem-like cells of glioblastoma multiforme**

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Recent studies have demonstrated the existence of cancer stem-like cells with CD133 surface marker in glioblastoma multiforme (GBM). It is believed that these minor CD133(+) tumor cells possess more efficient DNA-repair function than the majority CD133(-) progeny cells and thus can survive the radiation therapy and/or chemotherapy and rapidly give rise to fatal tumor recurrence. To isolate CD133(+) GBM cells, we administered ionizing radiation to short-term tumor cell cultures from 8 new GBM patients (participants in a clinical trial of immunotherapy). In one particular case, the GBM cells that survived irradiation contained 50- 60% highly clonogenic and neurosphere-forming CD133(+) cells. We also used FACS to isolate the CD133(+) cells from un-irradiated original GBM cell population of this patient. Karyotype analysis showed that the majority CD133(-) cells of initial culture were 46, XY, +7, +der(7;9)(p10;p10), -10, -18, der(19)(q13), with little change in near diploid karyotype within 10 passages *in vitro*. In contrast, majority of FACS-isolated un-irradiated CD133(+) cells showed a hypo-tetraploid with variation of chromosome number among them. The radiation-survived CD133(+) cells of this patient showed similar hypo-tetraploid patterns with slightly higher variation in chromosome number than the un-irradiated CD133(+) cells. Individual cell clones of the two CD133(+) categories were consistently hypotetraploid but still with variation of chromosome number, suggesting chromosomal instability during cell proliferation. Using these hypotetraploid CD133(+) GBM cells, we have obtained preliminary results that certain therapeutic agents that down-regulated CD133(+) expression also could shift the hypotetraploid to the near diploid karyotypes of CD133(-) GBM cells.

**P04.131****Familial Cancer Database (FaCD) online****R. H. Sijmons:***University Medical Center Groningen, Groningen, The Netherlands.*

Cancer is associated with a wide range of hereditary disorders. Recognising these disorders in cancer patients is important for the medical management of both patients and their relatives. The Familial Cancer Database (FaCD) - online is a web-based application aimed at healthcare professionals with at least basic knowledge of clinical cancer genetics. It has been developed to support the clinical genetic differential diagnosis in cancer patients and families. FaCD tries to match the tumour and non-tumour features observed in a particular patient and family with those of the disorders included in its database and provides a clinical synopsis with literature references for each of these disorders. In addition to reported familial clustering of cancer (e.g. familial cases of cervical cancer) and known cancer-associated hereditary disorders (e.g. Lynch syndrome), the database includes patterns of multiple primary tumours and data on the tumours associated with common multifactorial disorders (e.g. diabetes) or exogenous risk factors (e.g. use of OAC). In total, FaCD contains files on more than 400 different disorders. Users can choose from over 900 clinical tumour and non-tumour features to compose a search profile. Links are provided to corresponding OMIM entries, PubMed abstracts and websites of research and support groups. The database can be found at [www.facd.info](http://www.facd.info) and access is granted free of charge.

**P04.132****Quantification of CEA mRNA for micrometastases detection in peripheral blood and bone marrow specimens of gastric cancer patients by Real-Time PCR****L. Dardaei Alghalandis<sup>1,2</sup>, R. Shahsavani<sup>2</sup>, S. H. Ghaffari<sup>2</sup>, E. Aslankoooh<sup>2</sup>, K. Alimoghadam<sup>2</sup>, A. Ghavamzadeh<sup>2</sup>:**<sup>1</sup>Tarbiat Modares University, Tehran, Islamic Republic of Iran,<sup>2</sup>Hematology, Oncology & BMT Research center, Shariati Hospital, Tehran, Islamic Republic of Iran, <sup>3</sup>Khatam University, Tehran, Islamic Republic of Iran.

**Introduction:** Gastric adenocarcinoma is the first leading fatal malignancy in Iran. Despite advances in therapeutic approaches for gastric cancer (GC), tumor dissemination to distant organs is still the major cause of death. CEA that is a tumor antigen is abundantly expressed by malignant cells. The aim of our research was to use CEA for detection of micrometastases in peripheral blood (PB) and bone marrow (BM) specimens of patients with GC.

**Materials and Methods:** we used CEA as a tumor marker and GAPDH as an internal control to detect and quantify disseminated tumor cells in PB and BM specimens of affected individuals. Total RNA was extracted from AGS cell line and CEA and GAPDH fragments were generated by reverse transcription. The amplified fragments were cloned into T/A vector. Double cloning of these fragments has been done into T/A vector. Serial dilutions of this plasmid are used as standard curve, each containing a known amount of input copy number. Total RNA was extracted from PB and BM specimens of about 30 patients. cDNA of these specimens were synthesized by reverse transcription.

**Results:** We set up quantitative Real-Time PCR for CEA and GAPDH. The assay determined the copy number of disseminated tumor cells in the PB and BM specimens of patients.

**Conclusion:** The quantitative real-time PCR for the CEA can be a useful technique for detection of micrometastases in the PB and BM specimens of GC patients

**P04.133****DNA methylation of human telomerase reverse transcriptase (hTERT) gene in premalignant cervical lesions****P. Oikonomou<sup>1</sup>, A. Tsezou<sup>1,2</sup>:**<sup>1</sup>University of Thessaly, Larissa, Greece, <sup>2</sup>Institute of Biomedical Research and Technology, Larissa, Greece.

Human Telomerase Reverse Transcriptase (hTERT) mRNA expression seems to play an important role in cervical carcinogenesis. Analysis of the hTERT promoter region revealed the presence of a CpG island and a high overall GC content, suggesting a possible role for methylation in the regulation of hTERT gene expression. In the present study we evaluated the role of hTERT promoter methylation in hTERT regulation in premalignant cervical specimens. The methylation status of hTERT promoter gene, hTERT mRNA quantification and telomerase activity

were investigated in 26 normal and 64 specimens of abnormal cytology using the Methylight technique, the Telo TAGGG hTERT Quantification kit and LightCycler technology as well as Telomeric Repeat Amplification Protocol (TRAP). E6/E7 HPV-16 mRNA expression was also evaluated. No significant correlations were observed between hTERT mRNA expression and hTERT promoter methylation, as well as between telomerase activity and hTERT promoter methylation in normal and in premalignant cervical specimens. E6/E7 HPV-16 mRNA expression was observed in 72% of HPV-16 infected samples and was correlated with hTERT mRNA expression and telomerase activity ( $p<0.05$ ). This is the first study investigating the role of hTERT promoter methylation in hTERT mRNA expression and telomerase activity in premalignant lesions. The observed lack of correlation suggests that other mechanisms might be involved in the regulation of hTERT expression. The correlation between hTERT mRNA and E6/E7 mRNA expression confirms the role of HPV infection in hTERT regulation.

**P04.134****Identification of new splicing isoforms of the β-chimaerin gene by transcript analysis in human cancer tissues and cell lines****L. Barrio-Real<sup>1,2</sup>, M. Caloca<sup>2</sup>, R. González-Sarmiento<sup>1,2</sup>:**<sup>1</sup>Unidad de Medicina Molecular. Universidad de Salamanca, Salamanca, Spain,<sup>2</sup>Centro de Investigación del Cáncer, Salamanca, Spain.

Chimaerins are a family of GTPase-activating proteins (GAPs) that selectively inactivate the Rac GTPase. The four members of this family ( $\alpha 1$ -  $\alpha 2$ -  $\beta$ - and  $\beta 2$ - chimaerins) are generated by alternative start of transcription of two different genes, the  $\alpha$  and  $\beta$ -chimaerin genes. Chimaerin proteins have a catalytic GAP domain, a C1 domain that binds DAG and phorbol esters, and a SH2 domain that is only present in  $\alpha 2$  and  $\beta 2$ -chimaerin isoforms.

The  $\beta$ -chimaerin gene is located in the 7p15 locus and consists of 13 exons. In addition to the initial start site in exon 1, it possesses an alternative start of transcription in intron 6 that is used to render the  $\beta 1$ -chimaerin isoform. This alternative transcript has only been reported in rat testis.

Since recent work demonstrates a role for the  $\beta 2$ -chimaerin protein in cancer progression, we decided to perform a transcript analysis of the  $\beta$ -chimaerin gene in human cancer tissues and cell lines, to search for tumor-related alterations.

We report for the first time a differential pattern of expression of  $\beta 2$ - and  $\beta 1$ -chimaerin in several glioblastomas and tumor cell lines. In addition we have identified and characterized additional new isoforms generated by alternative splicing: 8 isoforms of  $\beta 2$ -chimaerin and 9 of  $\beta 1$ -chimaerin. Some of these isoforms maintain some functional domains, but most of them would render non-functional proteins which could behave as dominant negative mutants.

Functional studies of these new isoforms will help us to understand the mechanism of action of these proteins and their role in tumor development.

**P04.135****Chimerism monitoring: bone marrow vs. peripheral blood****A. D. Krstic<sup>1</sup>, O. Stojkovic<sup>2</sup>, M. Guc-Scekić<sup>1</sup>, D. Vujić<sup>1</sup>, D. Jevtić<sup>1</sup>, T. Varljen<sup>2</sup>:**<sup>1</sup>Mother and Child Health Care Institute Vukan Cupic, Belgrade, Serbia and Montenegro, <sup>2</sup>Institute of Forensic Medicine, School of Medicine, Belgrade, Serbia and Montenegro.

The goal of post-transplantation monitoring in hematopoietic stem cell transplantation (HSCT) is to predict disease relapse, graft rejection and graft-versus-host disease. The main principle in chimerism detection is to determine genetic markers which differ between donor and recipient and monitor them after transplantation with highest accuracy and sensitivity.

First it is important to determine whether engraftment took place and second whether the recipient cells, if present, are normal or malignant. Sensitivity of chimerism monitoring using STR-PCR analysis is 1-5%, and on the other hand the sensitivity of RT-PCR for minimal residual disease (MRD) monitoring is at least 1000 times higher.

We present three cases with detected genetic aberrations before HSCT: one patient had AML with complex karyotype 48,XX, t(11;17)(q23;q21), +6,+mar and two patients had ALL with 46,XX, t(9;22)(q21;q12) and bcr-abl (p190) rearrangement detected on molecular level. These patients were followed by cytogenetics and molecular genetics using BCR-ABL probes for MRD and STR markers for chimerism monitoring.

Our experience show that it is useful to follow up MRD in the patients bone marrow cells in cases where genetic aberrations were detected before HSCT, in order to be ready for appropriate intervention. In this work we discuss different chimerism status registered in the bone marrow and peripheral blood at the same time point, in cases with complications after HSCT.

#### P04.136

#### Cisplatin inhibits *in vivo* mouse telomerase activity in melanoma squamous cells

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Cis-diamminodichloroplatinum is a platinum (II) complex with chemotherapeutic use in treating neck and head cancers, small cell lung cancer, ovarian and testicular cancers. It is accepted that its mechanism of action relies on the fact that it binds to one DNA chain, by two guanosine residues, modifying DNA's double-chain structure and capacity to repair. The cell dies from apoptosis. Telomerase is a reverse transcriptase that adds TTAGGG repeating sequences to the 3' end of DNA strands in the telomere regions, which are found at the ends of the eukaryotic chromosomes. This way, it protects the genetic information in the DNA molecule by wasting with each replication cycle. Telomerase carries its own RNA molecule, used as a template for elongating telomeres. Cancer cells are expressing telomerase activity, while the most of the somatic normal cells do not.

We induced skin cancer in mice, by injecting them with dexamethasone and irradiating them with an UVB lamp. The mice were divided in two groups. One group was not treated. The other group was treated with cisplatin. Telomere lengths were analyzed in squamous cells from the carcinomic tissue of these mice by using the PCR / STELA technique. We found that the cisplatin treatment gradually reduces the telomere lengths in mice treated with this compound vs mice untreated, by decreasing telomerase's activity. We conclude that our findings support the idea that platinum compounds may have other ways of action in the cell nucleus, beside the classical one accepted.

#### P04.137

#### Quantification of CK20 mRNA for micrometastases detection in peripheral blood and bone marrow specimens of colorectal cancer patients by Real-Time PCR

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Introduction: Colorectal carcinoma is the third cause of cancer related deaths in the world. Despite advances in therapeutic approaches for colorectal cancer , tumor cells dissemination to distant organs is still the major cause of death.

Cytokeratins , are abundantly expressed by epithelial cells.The presence of epithelial cells in peripheral blood (PB) or bone marrow (BM) indicates the malignant nature of them. The aim of our research was to use CK20 for detection of micrometastases in PB and BM specimens of patients with colorectal cancer .

Materials and Methods:We used CK20 as a marker and GAPDH as an internal control. Total RNA was extracted from Caco2 cell line. CK20 and GAPDH cDNA were synthesized. Double cloning of these fragments has been done into T/A vector. Serial dilutions of this plasmid will be used as standard curve.Total RNA was extracted from PB and BM specimens of 30 patients, and cDNA were synthesized. we are quantifying CK20 mRNA levels and CK20/GAPDH mRNA ratios using a TaqMan real-time PCR system in the specimens.

Results: We set up quantitative PCR for CK20 and GAPDH using Real-Time PCR. The assay determined the copy number of disseminated tumor cells in the specimens of patients.

Conclusion: The quantitative real-time PCR for the CK20 can be a use-

ful technique for detectionof micrometastases in colorectal cancer.

#### P04.138

#### Screening of CYP1B1 alleles shows a less frequency of hyperactive variants Leu432Val and Asn453Ser in Mexican Population

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Human cytochrome P4501B1 is an important enzyme in the activation of diverse procarcinogens. CYP1B1 gene is polymorphic and hyperactive variants can lead to a higher susceptibility to estrogen-related cancers. Several single nucleotide polymorphisms have previously been reported in the human CYP1B1 gene. Previous studies have revealed a relation between Ala119Ser allele and breast cancer and between Leu432Val and prostate cancer, two polymorphisms that result in a higher catalytic activity of the enzyme. Mexican population shows a lower incidence of this type of cancers in comparison with other western countries. In the present study we analyzed the frequency of five CYP1B1 polymorphic sites to initially explore the possible relation between the lower incidence of estrogen-related cancer and the presence of these alleles in Mexican population. The genetic distribution of CYP1B1 variants was evaluated in 100 Mexican healthy subjects through genomic DNA sequencing analysis. The frequency of homozygous hyperactive variant Ala119Ser (23%) was higher than those reported in other populations. The frequencies of polymorphic variants 4326C>G (Leu432Val) and 4390A>G (Asn453Ser) (3% and 0%, respectively) were significantly lower than those observed in other populations except for Japanese population. The homozygous variant 4390A>G was not identified in our population. our data demonstrates that the CYP1B1 alleles encoding hyperactive variants, And the silent polymorphism 1347C>T, are significantly less frequent in Mexican population than those observed in western countries. Further studies should focus on the potential interaction of the occurrence of these polymorphisms and the incidence of estrogen-related cancers in Mexican population.

#### P04.139

#### Molecular characterization of patient-derived melanoma cell lines employed in therapeutic vaccine preparation

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We studied eleven human cutaneous melanoma cells lines with the aim to detect the presence of genetic aberrations currently considered to have a role in the pathogenesis of melanoma. By multiplex ligation dependent probe amplification assay (MLPA), mapping the region 9p21, we identified homozygous deletion of CDKN2B region in seven cell lines and homozygous deletion in CDKN2A region in eight cell lines. Sequencing analysis of the three cell lines that presented either loss of heterozygosity or no deletion for all loci tested in the 9p21 region showed deleterious mutations in two of them. NRAS and BRAF sequencing revealed a mutation in NRAS gene in one cell line and V600E change in BRAF in six cell lines. Four of the BRAF mutated cell lines presented one or more changes in MC1R. NRAS and BRAF mutations were mutually exclusive. No mutation affects CDK4 gene. We also have studied gains in several oncogenes by MLPA. NRAS (1p13) was amplified in the cell line carried NRAS mutation; CDK6 (7q21) was amplified in other seven cell lines. Amplifications of the chromosomal region 12p13 (CCND2) and 20q13 (STK15) were the more frequently detected (45% and 45% of cases, respectively). The region 11q13 (CCND1) was amplified in two cell lines. Finally, punctual amplifications of some oncogenes were identified (p.e. BIRC5). We detect two profiles: CDKN2A alterations with and without NRAS/BRAF/MC1R changes. Both patterns were associated to oncogenic amplifications. In conclusion, our results constitute a comprehensive molecular characterization of melanoma cell lines, offering important

information for melanoma research.

#### P04.140

#### A semi-automated unbiased differential methylation screening assay for applications in cancer epigenetic research

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Screening for differential methylation in tumor and control samples is one of the powerful approaches towards the identification of novel cancer related genes. Still there is no universal technique to screen for differential methylation, although the spectrum of methylation detection methods is vast. We have developed a synthetic highly effective, unambiguous, unbiased, semi-automated screening technique to serve as a possible basis for cancer epigenome research. Our differential methylation screening approach is based on a fluorescent methylation sensitive PCR with primers complementary to the adaptors ligated to the DNA digestion fragments. These AIMS (amplification of intermethylated sites) products are analyzed on automatic genetic analyzers with single-nucleotide resolution using GeneScan software. Specific software for automatic identification of differentially methylated fragments have been developed as well as the software capable of identifying the AIMS fragments *in silico* by comparison with available genome databases. Additional computer service is available to assist in experimentation design respective to specific applications. With technical variations this approach may be elaborated to identify novel targets of DNA methylation/demethylation as well as to roughly characterize epigenomic status as a whole. We suggest that the assay is universal and can be applied for screening differential methylation in any disease where epigenetic component may be suspected. *The study is supported by Friends for an Earlier Breast Cancer Test Foundation, USA.*

#### P04.141

#### The investigation of tumor suppressor gene (P53) LOCUS-17 P13 in late stage Endometriosis patients by fluorescent in Situ Hybridisation (Fish) technique

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In the study, the incidence of genetic alterations in p53 gene locus in late stage endometriosis cases were investigated in comparison with the endometrium and peripheral blood samples of the same patients and the same tissues of normal healthy women.

The study group was composed of six endometriosis samples (n:6). Control group included; normal endometrium samples (n:6, control I) and peripheral blood lymphocytes (n:6, control II) of the same patients with normal endometrium samples (n:5, control III) and peripheral blood lymphocytes (n:5, control IV) of the healthy women.

Frequency of monosomic, disomic and trisomic cells in controls and endometriotic tissue specimens were determined by using Multicolor Fluorescence in situ hybridization (FISH) technique and statistically analysed by Chi squared test.

In endometriosis samples the frequency of monosity and trisomy were 16.4 % and 2.1 % respectively for chromosome 17 p13 (p53) locus. In endometrium samples (n:6) the frequency of trisomy was 3.2 %. In peripheral blood lymphocytes (n:6) the frequency of trisomy was 0.3 % for the same locus.

Finally, chromosome 17p13 (p53) locus genetic alterations were found to be significantly greater ( $p<0.0001$ ) in the endometriosis specimens than in normal endometrial cells and peripheral blood lymphocytes of the same patients and healthy control group.

These findings support a multistep pathway involving somatic genetic alterations in the development and/or progression of endometriosis and give evidence for the need of more detailed, further studies on the subject.

#### P04.142

#### Evaluation of mtDNA common deletion ( $\Delta 4977$ ) in patients affected by esophageal SCC

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**Background:** Mitochondrial defects have long been suspected to play an important role in the development and progression of cancer. One of the most common mutations of mtDNA is a 4977 bp deletion that is called common deletion or  $\Delta 4977$ . The studies show that there is an association between this deletion and some cancer, aging and environmental factor such as alcohol and smoking. The aim of this study was to determine the frequency of  $\Delta 4977$  in mtDNA of tumor tissue in patients affected by esophageal squamous cell carcinoma.

**Methods:** The presence of  $\Delta 4977$  was investigated in mtDNA of 41 tumor tissues obtained from patients affected by esophageal squamous cell carcinoma, 10 adjacent normal tissues obtained from same patients and 10 blood sample of healthy individuals, using nested PCR standard protocol.

**Result:**  $\Delta 4977$  were detected in 33 out of 41 (80.5%) of tumor tissue, 9 out of 10 (90%) of adjacent normal tissues of same patients and none of the blood sample of healthy persons.

**Conclusion:** Our results demonstrate that  $\Delta 4977$  is prevalent in the esophageal squamous cell carcinoma and there is an association between this deletion and esophageal SCC ( $p<0.01$ ). This deletion may play an important role in the development and progression of esophageal.

#### P04.143

#### SDHD and SDHB: The clinical presentation,Phenotype and Care Pathway

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Familial Paraganglioma are rare tumours of the head and neck; they are often bilateral and are known to be more aggressive than spontaneous forms. The parasympathetic paraganglia lie along the whole of the parasympathetic nerves, paraganglioma are usually found at the intersection of large vessels. It has been shown that early detection of paragangliomas (PGs) has reduced morbidity following surgical intervention.

Clinical genetics have worked closely with endocrinologists in the 3 teaching hospitals in Manchester over the past 5 years with families with hereditary endocrine conditions including MEN type 1 and 2 and also Familial Paraganglioma syndrome (FPS). Due to the increased recognition of the syndrome and the advent of molecular testing the referral for assessment of paraganglioma / pheochromocytoma has increased exponentially.

To date 19 families with proven FPS have attended the Manchester Regional Genetics service.

We will describe the clinical presentation of families with SDHB and SDHD mutations, in particular the difference in the phenotype, the impact on the families and the care pathways for individuals both affected and at risk.

#### P04.144

#### Recent studies into the FA/BRCA pathway

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Fanconi anemia (FA) is a rare recessive disease with patients suffering from various congenital malformations, progressive bone marrow failure and high cancer susceptibility. Underlying FA are defects in any one of currently 13 known genes. Their products interact in the FA/BRCA pathway to mediate ICL repair involving NER, translesion synthesis and homologous recombination. This signalling path-

way consists of three major components: the nuclear core complex composed of at least eight FA proteins (group 1), which activates the "group 2" proteins FANCD2 and FANCI (ID complex). The ID complex is then targeted to chromatin and co-localizes with "effector" proteins such as BRCA2 or RAD51 (group 3). Recent progress in understanding the FA/BRCA network was achieved by the discoveries of the three FA genes *FANCI*, *FANCJ* and *FANCN*. Besides FANCD2, FANCI is the second monoubiquitinated member of the FA/BRCA pathway. We show the biallelic mutations of four FA-I patients. The 5'-3' helicase BRIP1 and the BRCA2-binding protein PALB2 both act downstream of the monoubiquitination step and belong to the "group 3" proteins. We identified biallelic truncating *BRIP1* mutations in eleven FA patients. In an additional seven FA patients we found biallelic *PALB2* mutations, designating *PALB2* as *FANCN*. Interestingly, investigations of *FANCJ* and *FANCN* have shown that all known group 3 proteins (BRCA2/FANCD1, BRIP1/FANCJ, PALB2/FANCN) are breast cancer susceptibility genes. This recent emergence of new FA genes not only facilitated further understanding of a major DNA repair pathway, but also makes connections to tumorigenesis and cellular aging (Neveling et al., Z Gerontol Geriatr 2007).

#### P04.145

#### **Role of Helicobacter Pylori in gastric adenocarcinoma : Special emphasis on gender, androgen receptor and angiogenic factors**

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*H. pylori* increase the risk of gastric cancer which has higher incidence in men globally. Due to animal studies male gastric tissues respond more rapidly to *H. pylori* but the underlying roles of sex hormones and angiogenic factors remained unclear in both genders. We studied the roles of *H. pylori* on tissue levels of Androgen Receptor (AR), uPA, MMP9 and TP as three major angiogenic and prognostic markers in gastric cancer. Malignant and non malignant tissues of 72 gastric adenocarcinoma cases who underwent surgery from 2004-2007 were analyzed by immunohistochemical methods. Higher prevalence of *H. pylori* in males, lack of AR expression in normal cells of females and lower expression of AR in tumoral cells of females ( $p=0.03$ ) were the first significant differences between two genders. *H. pylori* infection was evaluated as a relative risk factor of AR expression in males (OR=5) and females (OR=3) that means most of AR (+) tumors had history of *H. pylori* infection in their normal cells. Although females showed higher uPA expression ( $p=0.007$ ) but *H. pylori* negative tumors showed higher risk of tumoral uPA (OR=1.13), MMP9 (OR=1.24) and TP expression (OR=1.12) in males. By recording the strong role of AR and *H. pylori* infection on the expression of tumoral uPA and other angiogenic factors in women (OR=11) we concluded that *H. pylori* plays inconsistent roles in two genders by inducing different unknown genes but AR plays similar roles in both genders by increasing the tissue levels of angiogenic factors .

#### P04.146

#### **High frequency of MLH1 and MSH2 mutations among familial gastric cancer patients**

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Mutations of CDH1 gene are associated with familial diffuse gastric cancer. MLH1 and MSH2 mutations are associated generally with hereditary non-polyposis colon cancer syndrome that may include gastric cancer. So MLH1 and MSH2 mutations predispose to gastric cancer. However a frequency of MLH1 and MSH2 mutations among patients with familial gastric cancer unselected on cancer type or familial history is unknown.

We investigated the sample of 30 patients with familial gastric cancer on mutations of CDH1, MLH1 and MSH2 genes. The structure of our sample was near to a distribution of different familial gastric cancer types in the population and includes 8 families with diffuse gastric cancer. There were 5 germline MLH1 and MSH2 mutations among 30 patients (16,6%). Mutations have been found in families with gastric cancer only as well as in the families with both gastric and colon can-

cer cases. There were no mutations in CDH1 gene. Thus, a frequency of MLH1/MSH2 mutations associated with familial gastric cancer is higher in comparison with the CDH1 gene mutations ( $P=0.026$ ).

#### P04.147

#### **Telomere function in giant cell tumors of bone**

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**BACKGROUND:** Giant cell tumor of bone (GCT) is a locally aggressive tumor accounting for 1/20 of all bone tumors. Morphologically, GCTs show osteoclast-like giant cells mixed with mononuclear putative neoplastic cells. Cytogenetically, GCTs often show telomeric associations (tas) paralleled by an otherwise normal karyotype. It remains to be established whether tas in GCT represent deregulation of *cis*-regulatory or *trans*-regulatory mechanisms at the telomere, or a combination thereof, or are caused by other yet unknown mechanisms.

**METHODS:** DNA was extracted from fresh frozen biopsies of 20 GCTs for telomere length analysis. From the same 20 cases, RNA was extracted for quantitative RT-PCR analysis concerning the expression levels of four genes (*TERT*, *TRF1*, *TRF2* and *POT1*) involved in telomere function. FISH analysis was performed to evaluate the frequency of telomere-negative chromosome ends.

**RESULTS:** No correlation between presence of clonal chromosome aberrations and telomere length in tumor cells could be detected. At FISH analysis there was a correlation between frequency of tas and number of telomere-negative chromosome ends. Expression of *TERT*, *TRF1*, *TRF2* and *POT1* was found in all tumors. Analysis regarding the correlation between expression levels of individual genes and cytogenetic and clinical features is ongoing.

**CONCLUSION:** In view of our preliminary data, it is unlikely that the high frequency of tas could be explained by altered expression levels of any of the investigated genes. However, it can not be ruled out that altered function at the protein level of *TERT*, *TRF1*, *TRF2* or *POT1* is involved in tas formation.

#### P04.148

#### **Overexpression of wild-Type p53 suppresses Cathepsin B (CSTB) in glioblastoma cells**

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The tumor suppressor protein p53 plays critical role in modulating cellular functions such as cell cycle arrest and apoptosis. Due to its function in growth inhibition as well as contribution of its mutated form in >50% of human tumors particularly a significant proportion of glioma cases, p53 is considered a fundamental tumour suppressor gene. In order to investigate potential p53 target gene(s), we employed overexpression of wild-type p53 via recombinant adenovirus (Ad-GFP-P53) which encodes green fluorescent protein and p53 separately, and cDNA AFLP approaches in U87 glioblastoma cells. In response to overexpression of wild-type p53, cDNA AFLP results revealed the suppression of *cathepsin B* (CSTB) gene which encodes a protease but not in infected cells with Ad-GFP which does not express p53 and mock control. Semi-quantitative RT-PCR analysis confirmed the activation of *CTSB* gene in cells containing Ad-GFP and mock control however; its transcriptional activity was suppressed in infected cells with Ad-GFP-P53. In addition, computational analysis detected several potential p53 DNA target sequences within introns of *CTSB* genes. Taken together our results suggested that *CTSB* gene might be directly regulated by p53 protein as a transcription regulatory protein in glioblastoma.

#### P04.149

#### **MGMT and p15 promotor methylation - prognostic parameters for TMZ chemotherapy response?**

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Glioblastomas are the most frequent and malignant brain tumors in adults. Surgical cure is virtually impossible and despite of radiation and chemotherapy the clinical course is very poor. Epigenetic silencing of

MGMT has been associated with a better response to TMZ-chemotherapy. We were also able to show that TMZ increases the median survival time of patients with tumors harbouring deletions on 9p within the region for p15 and p16.

The aim of our current study was to investigate the methylation status of p15, p16, p14<sup>arf</sup> and MGMT and correlate these results with the clinical data.

DNA was isolated from fresh frozen GBM biopsies (n=27) and modified by sodium bisulfite. Promotor methylation of p15, p16, p14<sup>arf</sup> and MGMT was analyzed by MS-PCR. Only patients with a KPS >70, radiation and TMZ-chemotherapy after radical tumor resection were included.

We observed promotor hypermethylation of MGMT in 56%, and of p15 in 37% of the tumors, whereas hypermethylation of p16 and p14<sup>arf</sup> were rare.

Interestingly, hypermethylation of p15 emerged as a significant predictor of a shorter overall survival (16,9 vs. 23,8 months, P=0,025; Log-rank test), whereas MGMT hypermethylation had no effect on median overall survival (22,5 vs. 22,1 months, P=0,49; Log-rank test). In the presence of other clinically relevant factors (age, KPS, sex, MGMT, p15 hypermethylation remains the only significant predictor (P=0,021; Cox regression).

Although these results need to be confirmed in larger series, our retrospective study suggests that p15 hypermethylation can act as an additional prognostic factor for survival in glioblastomas.

#### P04.150

##### Genetic basis in Gorlin Syndrome

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Gorlin syndrome is an autosomal dominant disorder characterized by multiple developmental abnormalities and predisposition to suffer tumours. SHH receptor gene Patched1 has been directly related to Gorlin syndrome. It represses Smoothened, which transduces SHH signal to activate transcription factor GLI family.

We have analyzed PTCH1 gene in 64 unrelated patients with Gorlin syndrome, using PCR, heteroduplex and automatic sequencing. We found 25 different mutations, 10 of them are reported for the first time: IVS2(-2)delAGTTGGAGGACGAGTA; IVS5(-56) T>G; IVS6(+1)G>A; IVS11(+20)delGAG; g.417insA; g.726delCA; g.1421insG; p.W851X; p.P1050P and g.2517delC. We also searched for PTCH in 38 patients by MLPA. We found 9 patients with amplifications and 3 cases with deletions. Moreover, in cases without abnormalities in the PTCH1 locus, we have analyzed new candidate genes in the SHH pathway: We found 3 mutations (p.H262H; p.R509W; p.R531C) in GLI1 gene. The 2 missense mutations have been screened in 200 healthy controls. R509W was found in one healthy subject, while R531C was not. GLI2 analysis showed new intronic and silent mutations. We found 3 new missense mutations in CDON gene (p.I75V; p.E162K; p.A686V) and 2 new missense changes in BOC gene (p.E915H and p.Q959K). All of them have been screened in 50 healthy controls resulting as polymorphisms. Suppressor of Fused, EGFR and KCTD11 genes do not present pathogenic mutations in patients with Gorlin syndrome. In conclusion, 20.3% of patients with Gorlin syndrome present mutations in PTCH1 gene. We also conclude R531C mutation in GLI1 seems to be pathogenic and responsible for Gorlin syndrome.

#### P04.151

##### Glutathione S-transferase (GSTM1) polymorphism in Serbian head and neck tumour patients

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Susceptibility to head and neck tumours in a particular individual may depend in part on the metabolic balance between Phase 1 enzymes, such as cytochromes P450 (CYPs), and Phase II enzymes, such as glutathione S-transferases (GSTs). Impaired detoxification of carcinogens found in tobacco smoke appears to increase the risk for tobacco associated cancers. Glutathione S-transferases (GSTs) are involved in

the metabolism of a wide range of carcinogenic chemicals. In humans, cytosol GSTs are divided into several classes, and polymorphisms of these enzymes are associated with variations in enzyme activity which in turn may affect the concentration of activated carcinogenic chemicals in the body. We have investigated the association between the polymorphism in one of the cytosolic GSTs gene (GSTM1) and susceptibility to oral squamous cell carcinoma (OSCC) and basal cell carcinoma (BCC) of the skin. For that purpose 45 SCC, 42 BCC and 40 control specimens have been subjected to PCR analysis of the GSTM1 gene. The frequency of GSTM null homozygous genotype was as follows: 48% in BCCs, 44% in SCCs and 20% in controls. The deletion of the GSTM1 gene was more frequent in SCC, than in controls (p=0.017) and the same was true for BCCs (p=0.016).

Although cytosolic glutathione S-transferase (GST) enzymes occupy a key position in biological detoxification processes, GSTM1-1 gene is deleted in a high percentage of the healthy human population, with major ethnic differences. We found a significant difference in genotype frequencies between the two tumour groups and healthy controls.

#### P04.152

##### Molecular Assessment of Chimerism after Allogeneic Hematopoietic Cell Transplantation: Clinical utility in monitoring cell lineage engraftment

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Allogeneic hematopoietic cell transplantation (HCT) is a treatment option for a variety of hematopoietic malignancies as well as nonmalignant diseases. Monitoring of chimerism by molecular techniques has been widely utilized in the routine clinical setting. Chimerism analysis is usually performed on DNA extracted from unfractionated blood or bone marrow samples. Analysis of lineage-specific leukocytes might be useful in predicting the clinical outcome and might play a critical role in the management of HCT patient. We have used an Analyte Specific Reagent on a Beckman capillary electrophoresis platform to assess the dynamics of chimerism within the individual leukocytes subsets in 83 hematopoietic cell transplant patients after myeloablative and nonmyeloablative regimens. Magnetic anti-CD3, anti-CD15 and anti-CD19 beads were used to capture T cells, myeloid cells and B cells, respectively, from peripheral blood and bone marrow samples prior to isolation of DNA. Twelve different polymorphic short tandem repeats (STR) loci were amplified in a single multiplex reaction. Chimerism analysis performed on specific cellular lineages was most useful in early detection of relapse than unfractionated whole blood analysis due to the increased sensitivity in the enriched cell subpopulations. Determination of the level of donor T cells appears to be critical for successful engraftment and could predict graft rejection.

#### P04.153

##### Hepatoblastoma: *in vivo* and *in vitro* models for the analysis of Wnt/β-catenin and IGFs pathways

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Hepatoblastoma (HB) is the most important paediatric liver cancer (1% in children aged 6mo-3yr). HB is a multi-factorial condition including both genetic and environmental factors.

Environmental factors may contribute to HB pathogenesis; exposure to phthalates (i.e. diethyl-2-hexyl-phthalate-DEHP), present in PVC-based devices, are suggested as a risk factor in the highly susceptible subpopulation of premature and low-birth weight newborns. DEHP interacts with nuclear receptors, thus interfering with a number of signalling pathways.

To investigate the role of phthalate exposure, animal and cell line studies are in progress to characterize the effects of DEHP, in comparison with benzofuran, a HB inducer in aged mice. Furthermore, histological and immuno-histochemical valuation will be performed on frozen and paraffin-embedded human tissues from HB patients. To elucidate molecular mechanism(s) of HB pathogenesis we will focus on *i*) the role of Wnt/β-catenin and IGF pathways, and *ii*) its possible early diagnostic

and/or prognostic markers, human cell lines and tissues from mice treated with DEHP and BF.

$\beta$ -catenin and APC gene mutational analysis, modulation of gene expression analysis, genomic post-transcriptional regulation analysis through microRNA and protein expression patterns are in progress. We assessed *i*) the *in vitro* cytotoxicity of DEHP and BF in the HB cell line HuH6, *ii*) the *in vitro* differential expression of two microRNAs, *iii*) the differential protein expression in some gene products of the Wnt/ $\beta$ -catenin signalling pathway.

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#### P04.154

##### Influence of the focal adhesion protein leupaxin on invasion and metastasis in TRAMP prostate carcinomas

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In a recent study we showed that leupaxin expression could be detected in 21% of human prostate carcinomas (PCa). Down-regulation of leupaxin expression using RNAi resulted in apoptosis of more than 50% of androgen-dependent LNCaP PCa cells and in a significant reduction of invasiveness and migratory ability of androgen-independent PC-3 and DU145 PCa cells.

To analyse the role of leupaxin in progression and invasion of PCa in vivo we bred transgenic mice expressing prostate-specific leupaxin with TRAMP mice to generate double transgenic heterozygous mice. The transgenic adenocarcinoma of the mouse prostate (TRAMP) model develops, as a consequence of transgene SV40 T/t antigen expression, progressive prostate cancer that is invasive and capable of metastatic spread. Histopathological analysis showed that at the age of 22 weeks 91% of double heterozygous mice display a poorly differentiated tumor. In addition, 65% of these mice developed metastasis to the lymph nodes. In single heterozygous TRAMP mice only 50% showed a well differentiated tumor while none of them developed metastasis. Furthermore, our recent studies could demonstrate that the expression of the cell-cell-adhesion molecule catenin delta 1 (p120CTN) negatively correlated with the expression of leupaxin. No expression of p120CTN could be detected in progressive and invasive prostate tumors of double transgenic TRAMP/leupaxin mice. In conclusion, the results of our studies indicate that leupaxin expression enhances the progression of PCa towards a more aggressive and metastatic tumor and that the loss of p120ctn expression due to leupaxin overexpression represents one possible cause of the invasive behavior.

#### P04.155

##### Does nonsense-mediated RNA decay support TP53 haploinsufficiency as the cause of tumour development in Li-Fraumeni syndrome?

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Li-Fraumeni syndrome (LFS) is characterised by predisposition to a broad spectrum of cancers. The syndrome is associated with germline TP53 mutations, usually missense. The aberrant protein produced from the mutated TP53 allele is expected to cause tumour development. However, a possibility remains that haploinsufficiency of the normal TP53 allele could also be responsible for the phenotype. We analysed two LFS families with nonsense germline TP53 mutations. Nonsense-mediated RNA decay (NMD) eliminates transcripts with premature termination codons (PTC). In LFS families with nonsense mutations NMD could thus influence phenotype, because the removal of mutated transcripts would lead to the absence of the aberrant protein. TP53 alleles were analysed in normal tissues (lymphocytes, adrenal gland) and in tumours of both families on DNA and RNA levels. In normal tissues the transcripts of mutated alleles were eliminated, similar to some of the tumours. This may support the notion that TP53 haploinsufficiency,

and not the mutated protein, could be responsible for tumour development in LFS. However, in some tumours both types of transcripts (or only mutated transcripts) were present. This could reflect either differences in NMD between different tissues, or global inactivation of NMD in tumours. In quest to distinguish between these possibilities we tested expression of several genes (U2AF35, RPL3, TM1, PTB2, PTB1, ABCC4) with PTC-containing alternative transcripts, which should undergo NMD under physiological conditions. However, these analyses were inconclusive, mainly because the patterns of removal of some transcripts described in the literature could not be replicated. Supported by grants MSM0021620813 and MZO00064203.

#### P04.156

##### Molecular basis of the Li-Fraumeni syndrome: an update from the French LFS families

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Extensive analysis of TP53, based on complete sequencing of the 11 exons and QMPSF analysis to detect genomic rearrangements, in 474 French families suggestive of LFS, as defined by the Chompret criteria, led us to identify in 82 families (17%) a germline alteration of TP53. Most of the alterations corresponded to missense mutations (67%) and we identified in 4 families (5%) genomic deletions. These results represent a definitive argument demonstrating that LFS results from TP53 haplo-deficiency. Nevertheless, this does not explain the remarkable TP53 germline mutation spectrum characterized by the predominance of missense mutations. The different tumour spectrum observed between TP53 wt/mt and wt/- mice led us to compare the mean ages of tumour onset between patients harbouring either TP53 missense mutations or other types of alterations. We found a significant difference, missense mutations being associated with a 9-year earlier tumour onset, confirming that missense mutations not only inactivate p53 but also have an additional oncogenic effect. The observation of a later age of tumour onset associated with null mutations led us to perform TP53 analysis in patients suggestive of LFS, but with a first tumour being diagnosed after 40 years of age and we had the surprise to identify germline TP53 mutations in such families. Germline alterations of TP53 that lead exclusively to loss of function are therefore associated with a later age of tumour onset and the presence of such mutations should be considered in atypical LFS families with tumours diagnosed after 40 years.

#### P04.157

##### Genetic analysis of tumor cell-free DNA in patients with lung cancer

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Cell-free DNA (cfDNA) circulating in plasma was found in healthy individuals, but its concentration can increase in patient with cancer. In these, total plasmatic cfDNA is the mixture of DNA from normal and tumor cells. Genetic analysis of tumor cfDNA in plasma could be a non-invasive tool for early diagnostics and other study of pulmonary tumors. The concentration of total cfDNA was measured by quantitative real-time PCR. We analysed total cfDNA by using time-release PCR with primers for short tandem repeats (STR) located at tumor suppressor genes TP53, APC, and FHIT: diTP53, D5S346, D5S318, D5S299, D5S82, D3S1300, D3S1560. We have studied loss of heterozygosity (LOH) or microsatellite instability by using capillary electrophoresis. DNA from peripheral nucleated blood cells from the same patient served as a control. We have analysed cfDNA of patients with lung cancer (n = 33), with other pulmonary non-tumor diseases (17), and control group of healthy individuals (29). We have proved simultaneous presence of LOH in multiple STR loci in majority of lung cancer patients in contrast

to both control groups; therefore, the presence of multiple LOH in a sample of cfDNA could predicate lung cancer. LOH of D5S299 had the highest sensitivity, and D5S346 (both in the vicinity of APC gene) had the highest specificity of all used primers. In the lung cancer group, there were two patients where one of two alleles of D5S82 or D5S318, respectively, completely disappeared; this was not seen in any individual in both control groups.

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#### P04.158

#### M6P/IGF 2R is mutated in human endometrial adenocarcinomas and lung cancer

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The M6P/IGF 2 receptor has been shown to be mutated in a number of human cancers and to suppress cancer cell growth, indicating it as a tumor suppressor. The aim of this study was to determine if the M6P/IGF 2R is a target for mutation in human endometrial and lung cancers, tumors where perturbation of level of IGF's peptides seems to be implicated in neoplastic growth by autocrine/paracrine mechanisms. The tumors were analyzed for loss of heterozygosity (LOH). In those with LOH at one M6P/IGF 2R locus, the remaining allele was screened for mutation in ligand binding region by direct sequencing of PCR products. Among 10 informative adenocarcinoma samples 4 had LOH at M6P/IGF 2R locus. Of them, two samples harbored the mutation in the remaining allele as well. Of 46 endometrial adenocarcinomas 16 had LOH at one IGF 2R locus. The remaining allele in 8 of these samples contained also the mutation in the IGF 2 binding domain. As IGF 2R mediate activation of the growth inhibitor TGF- $\beta$  and clearance of growth promoter IGF 2, whose down and up regulation are involved in malignant transformation, it is reasonable to believe that dysfunction of IGF 2R by mutation, could also contribute to tumor development.

#### P04.159

#### Candidate gene screening for melanoma susceptibility

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Melanoma is a multifactorial and polygenic disease. The main risk factors are number of nevi, familial predisposition and skin phototype related to ultraviolet radiation exposition. Ten percent of cases are detected in a familial setting, being then inherited as an autosomal dominant trait. Two high susceptibility genes have been implicated: *CDKN2A/p14ARF* and *CDK4*. Mutations are detected in 20-60% of families. Nevertheless, sporadic melanoma accounts for 90% of cases. The molecular basis of melanoma predisposition in such patients has not been well characterized.

Aim: *i)* to determine the real implication of *CDKN2A* and *CDK4* in sporadic melanoma and to evaluate the clinical differences between mutation carriers and non-carriers. *ii)* Genotyping of 265 SNPs (located in 38 genes) to look for low susceptibility genes implicated in melanoma and nevus predisposition.

696 melanoma patients have been screened for *CDKN2A* and *CDK4* mutations. SNP genotyping has been carried out in 260 melanoma patients using the GenomeLab SNPstream Genotyping System. Sixteen sporadic Melanoma patients are *CDKN2A* mutation carriers (2.3%). Mutations are more frequent in multiple melanoma (MPM) than in single primary patients (SPM) (12.2% vs 1%, p=0.000). Age at onset is lower in mutation carriers than non-carriers (36.63 vs 50.63 y.o, p=0.001). A148T variant has been detected in 14.6% of patients with MPM and in 7.2 of SPM (p=0.02), suggesting that A148T could act as a low susceptibility allele to melanoma predisposition. Results of SNP genotyping are being analized and data will be discussed.

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#### P04.160

#### Germline mutations of *CDKN2A* in multiple and familial melanoma Brazilian kindreds

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**Background:** Melanoma is hereditary in approximately 10% of cases. Familial melanoma is described as a family in which either 2 first-degree relatives are diagnosed with melanoma, or families with 3 melanoma patients (irrespective of degree of relationship). *CDKN2A* is the first identified and the most important melanoma susceptibility gene. The mutation spectrum in the Brazilian population is unknown.

**Aim:** Report our experience in screening of mutations of *CDKN2A* gene in Brazilian kindreds with clinical diagnosis of Familial Melanoma. This study is part of the Melanoma Genetics Consortium, (GenoMEL) in Latin America.

**Methods:** Patients with multiple or familial clustering of Melanoma were genetically tested. Families were identified applying the GenoMEL diagnosis criteria. Point mutations in *CDKN2A* gene were screened by denaturing high performance liquid chromatography (DHPLC) followed by direct sequencing.

**Results:** Genomic DNA of 12 patients with clinical diagnosis of familial melanoma was obtained from peripheral blood samples. *CDKN2A* gene was divided into 9 fragments covering the 4 exons with substantial parts of intron regions. Blood sample was collected from one adult healthy control that not presented mutation in *CDKN2A* gene and it is used as a reference for DHPLC experiment.

#### P04.161

#### *MC1R* polymorphisms, phenotype and UVB radiation sensitivity in melanoma risk patients

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**Background:** UV radiation (UVR) plays an important role in melanocytic tumours development. History of intense intermittent sun-exposure, phenotypic characteristics encoding melanocortin-1 receptor gene (*MC1R*) and the presence of multiple nevi are risk markers to develop malignant melanoma. Familiar melanoma represents 10% of all melanomas and it is supposed that genetic burden in these patients plays a more important role than environmental factors.

**Objective:** to study in different melanoma-risk patient settings the association between phenotypic characteristics, number of nevi, UVR sensitivity and polymorphisms in *MC1R*.

**Methods:** two groups of high risk melanoma patients were studied (N=32):

Group 1: Dysplastic nevus syndrome (DNS) patients (N=23).

Group 2: Melanoma patients belonging to familial melanoma (N=9). UVB sensitivity, phenotypic and genotypic characterization were performed.

**Results:** patients from DNS had a higher UV-B photosensitivity (reduced UV-B minimal erythema dose) compared with patients from familiar melanoma group (mean 88mJ/cm<sup>2</sup> ± 26 vs 122 mJ/cm<sup>2</sup> ± 26; p<0.005). In both groups a high percentage of *MC1R* polymorphisms were detected (at least 1 polymorphism in 75% of patients in group 1 vs 78% in group 2). However, a different percentage of red hair type polymorphisms (RHP) was observed in the 2 groups (20% in group 1 vs 66% in group 2, p<0.01).

**Conclusions:** Patients affected by DNS presented high UV-B sensitivity, similar to North European populations or patients with photo-dermatoses. Similar percentage of *MC1R* polymorphisms were detected in our both samples, however, RHPs were strongly associated with familial melanoma, an unexpected result in our Mediterranean area.

**P04.162****HER-2/neu Amplification in Paraffin Embedded Tissue Sections of Meningioma Patients**

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Meningiomas arise from the meningoendothelial cells and are one of the most common tumors of the central nervous system. About 90% of meningiomas are benign. They are histologically classified as benign (Grade1), atypical (Grade2) and anaplastic (Grade3) according to World Health Organization (WHO) 2007 classification of brain tumors, which also correlates with disease prognosis. Numerical and structural chromosome abnormalities have been reported in meningiomas. HER-2/neu gene is located on 17q11.2-q12 chromosome region and encodes an epidermal growth factor receptor. HER-2/neu gene amplification and/or over expression have been detected in some human cancers and studied most widely in breast carcinomas. Previous studies have shown the importance of HER-2/neu gene amplification on the prognosis of meningioma cases. In this study, we aimed to detect HER-2/neu gene copy number in archive materials of 55 meningioma patients by fluorescent *in situ* hybridization (FISH). The patients included in the study have been operated in the neurosurgery department of our hospital between 1999 and 2002. Tissue samples were classified histologically according to WHO 2007 guidelines. We found HER-2/neu gene amplification in 7 (12,73%) patients. Another 2 patients had only one signal for the HER2-neu region. We confirmed this finding by a second hybridization with chromosome 17p13.1 (p53) probe. According to our results, HER-2/neu amplification could be regarded as an additional genetic factor playing role in meningioma pathogenesis together with known chromosomal abnormalities.

**P04.163****Methylenetetrahydrofolate reductase gene (MTHFR) 677C>T polymorphism in Serbian oral squamous cell carcinoma and basal cell carcinoma patients**

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Enzyme methylenetetrahydrofolate reductase (MTHFR) regulates intracellular folate metabolism through conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which in turn play an important role in cancerogenesis because of its involvement in DNA methylation and nucleotide synthesis. A common genetic polymorphism in the MTHFR gene - C677T is associated with thermolability and decreased enzyme activity. Reduced MTHFR activity may decrease the methylation of homocysteine to methionine, resulting in DNA hypomethylation. The contribution of MTHFR polymorphisms to the susceptibility to various types of cancer is controversial. We investigated a possible association of MTHFR polymorphisms 677C>T and increased risk for oral squamous cell carcinoma (OSCCs) and basal cell carcinoma (BCCs).

PCR- RFLP analysis of the MTHFR gene was performed on DNA obtained from 40 SCC and 35 BCC patients and 400 healthy individuals. The following genotype frequencies were found: 25% (CC), 67.5% (CT) and 7.5% (TT) in squamous cell carcinomas; 34.3% (CC), 57.2% (CT) and 8.5% (TT) in basal cell carcinoma patients. Genotypes in controls were: 40% (CC), 46 (CT)% and 14% (TT). A statistically significant difference ( $p<0.05$ ) was found in the genotype frequencies between the two tumour groups and the control.

**P04.164****MTR A2756G polymorphism confer a risk for colorectal cancer in Romanian patients?**

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**Introduction:** Epidemiologic and mechanistic evidences suggest that folate is involved in colorectal neoplasia. MTR, an essential enzyme in folate metabolism, catalyzes the remethylation of homocysteine to methionine. The A2756G polymorphism was implicated in modification

of plasma homocysteine level.

**Aim:** The goal of this study was to analyses the association between MTR A2756G polymorphism and colorectal cancer in Romanian patients.

**Means & methods:** Blood samples were obtained, after informed consent, from individuals with colorectal cancer (M:F=53:40), and healthy persons (M:F=55:45). Genomic DNA was extracted from peripheral blood leucocytes using commercial kits and the MTR A2756G polymorphism was assessed by PCR RFLP.

X<sup>2</sup> test has been used for statistical analysis ( $p<0.05$  was considered significant).

**Results:** This polymorphism respect Hardy-Weinberg equilibrium in both case and control groups ( $\chi^2=0,195$ ,  $p=0,658$ ;  $\chi^2=0,541$ ,  $p=0,461$ ).

The procentual distribution of A2756G AA:AG:GG in patients and controls was 63,4:33,4:3,2 and 67: 31:2 respectively. In our study, the frequency of genotype GG was appropriate that in other European series (approximately 3 percent).

For MTR polymorphism, distribution of genotype frequencies and allele frequencies don't differed significantly between cancer patients and control ( $\chi^2=0,269$ ,  $p=0,603$ ; respectively  $\chi^2=0,364$ ,  $p=0,54$ ).

**Conclusions:** In this study, we find no association between MTR A2756G polymorphism and colorectal cancer Romanian patients. Thus, this potential link must be evaluated between in much powerful studies.

**P04.165****Paraganglioma in a patient with multiple endocrine neoplasia type 1: Preservation of the MEN1 gene heterozygosity and detection of the somatic point mutation in the wild-type allele**

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominantly inherited disorder characterized by hyperplastic and neoplastic disorders in endocrine organs such as parathyroid, anterior pituitary and endocrine pancreas. Development of pheochromocytoma in patients with MEN1 is very rare and there have been only a handful of case reports. Paraganglioma in MEN1 has not been reported in English literature. Involvement of MEN1 gene in pheochromocytoma/paraganglioma has not been examined and molecular mechanism of development of such tumors remains elusive. Here we report a patient with MEN1 who developed retroperitoneal paraganglioma. The patient, a 63-year-old Japanese woman, was referred to us because of retroperitoneal tumor. She had been diagnosed as having MEN1 (primary hyperparathyroidism and pituitary tumor) and genetic testing had revealed a heterozygous mutation in exon 10 of the MEN1 gene (c1546-1547insC). The tumor was surgically resected and that, pathologically diagnosed as paraganglioma, was applied to genetic analysis. In addition to the germline mutation in exon 10, a somatic frameshift mutation (c1217delA) was identified in exon 9 of the MEN1 gene in DNA prepared from the tumor; this mutation was not seen in DNA prepared from peripheral mononuclear cells. Allele specific amplification and subsequent sequence analysis revealed that these two mutations exist on the different allele, indicating both alleles are functionally lost in tumor cells. This was further confirmed by immunostaining study. This is the first report which demonstrates causal relationship between inactivation of the MEN1 gene and development of paraganglioma.

**P04.166****High frequency of the heterozygous p.I171V mutation of the NBS1 gene in larynx cancer and multiple primary tumors**

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The higher incidence of multiple primary tumors (MPT) is a significant problem in head and neck tumor treatment. Recent studies suggest that carriers of heterozygous mutations in the NBS1 gene have an increased risk of malignant tumor development. The aim of our research was to assess the frequency of NBS1 mutations in patients with larynx cancer only (LC) and with MPT. The MPT group consisted of patients with one cancer localized in the larynx (primary or second)

and another at another site. DNA from 175 patients with LC and 104 patients with MPT was analyzed using the single-strand conformation polymorphism (PCR-SSCP) method and direct sequencing. We found 9 carriers of the p.I171V mutation among these 279 cancer patients and only 1 carrier among 500 population controls (0.2%). Four carriers of the p.I171V mutation were detected among 175 LC patients (2.3%) and 5 among 104 patients with MPT (4.8%). The frequencies of the p.I171V mutation carriers in LC and MPT patients were significantly higher than in controls (OR=11.7, CI 1.3-105.2, p=0.0175 and OR=25.2, CI 2.9-218.2, p=0.0007; respectively). In 1 individual with LC, a novel molecular variant c.1222A>G (p.K408E) was identified. No carriers of p.R215W or 657del5 NBS1 mutations were found in the present study. These findings imply that heterozygous carriers of the p.I171V mutation are prone to the development of larynx cancer and may, in addition, display an increased risk of second tumors at other sites.

#### P04.167

#### CpG island methylator phenotype in primary neuroblastomas is associated with poor survival

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Hypermethylation of CpG islands (CGIs) is an epigenetic phenomenon that contributes to carcinogenesis. We have determined the methylation pattern of several genes involved in distinct biological pathways in neuroblastoma (NB), one of the most common pediatric solid tumors. 82 primary NB tumors were studied by methylation specific PCR (MSP) for twenty genes. Three of them were excluded (MGMT, SYK and GSTP) because showed no evidence of promoter hypermethylation. CpG island methylator phenotype (CIMP) status was defined as hypermethylation of more than 50% from the 17 genes analyzed. We found that some genes as TMS1, CASP8, THBS1, CCND2, RASSF1A, BLU, EMP3 or DR4 are more frequently methylated in NB cases with a poor prognosis. CIMP status was significantly associated with disease stage ( $P=0.0002$ ), risk group ( $P=0.00004$ ), age at diagnosis ( $(P=0.0007)$ , N-myc amplification ( $P=0.005$ ) and 1p deletion ( $P=0.003$ ). By a Kaplan-Meier analysis, the 27 CIMP+ cases showed significantly poorer disease-free survival (DFS) ( $P=0.0084$ ) and overall survival (OS) ( $P=0.0062$ ) than the 55 CIMP- cases. However, Cox regression analysis failed to demonstrate that CIMP was an independent factor to the remaining variables, except for the clinical stage.

We conclude that CIMP status could strongly influence on OS in NB tumors, being a better prognosis factor than the other high-risk variables except for the disease stage. Thus the presence of CIMP may lead to a poor prognosis by induction of CGIs methylation.

#### P04.168

#### DCX expression in bone marrow after induction correlates with reduced survival in high-risk neuroblastoma patients

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**Background:** Detection of minimal residual disease (MRD) in bone marrow (BM) and peripheral blood (PB) is crucial for follow-up in high-risk neuroblastoma patients and might impact on survival.

**Methods:** Relative quantification of DCX and TH was studied by quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) using Assays on Demand from Applied Biosystems (Oltra et al., 2005, Diagn Mol Pathol. 14: 53-57). We studied DCX and TH expression in 87 high risk neuroblastoma patients (78 stage 4 and 9 stage 3) treated according cooperative national protocols in Spain.

**Results:** The frequency of DCX and TH detection at diagnosis in BM was 75,3% and 76,7%. After induction chemotherapy the frequency of both markers decreased to 37,8% and 28,9%. In PB samples the frequency of DCX and TH was 58,3% and 50%, respectively, at diagnosis and 12,5% and 15,6% after induction.

Only the DCX expression in BM after induction chemotherapy showed a statistically significant predictive value. Five years overall survival (OS) and event-free survival (EFS) were significantly reduced in patients with DCX expression in BM after induction ( $p<0.002$ ).

**Conclusions:** DCX expression in BM after induction chemotherapy showed a statistically significant impact on OS and EFS in high risk neuroblastoma patients. In contrast, DCX expression in BM or PB at diagnosis did not show a disease prognostic value. TH expression did

not show prognostic value in PB and BM at these treatment points.

#### P04.169

#### Long-term persistence of clonal TCR and/or BCR gene rearrangements in patients with Nijmegen breakage syndrome

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**Background:** Nijmegen breakage syndrome (NBS), caused by mutations in the *NBN* gene, is an autosomal recessive chromosomal instability syndrome associated with high cancer risk. The NBN protein is a component of the MRE11/RAD50/NBN complex involved in processing DNA double-strand breaks. It is therefore particularly important to study abnormal recombination events in NBS patients, given the inherited predisposition to develop lymphomas and leukemias.

**Objectives:** The aim of this study was a longitudinal analysis of T-cell receptor (TCR) and immunoglobulin/B-cell receptor (BCR) gene rearrangements in peripheral blood mononuclear cells (PBMC) in NBS patients, and their correlation with eventual lymphoma development.

**Patients and methods:** A total of 50 NBS patients (all carrying the 657del5 mutation on both alleles of the *NBN* gene) were periodically monitored for clonality of TCR and BCR gene rearrangements in PBMC by multiplex PCR-heteroduplex analysis. The same method was also used for molecular analysis of lymphoma tissues. Identity of clonal blood and/or tissue rearrangements was determined by direct sequencing.

**Results:** Seventeen NBS patients showed long-lasting clonal TCR and/or BCR rearrangements in PBMC, and identical clones persisted up to 4 years. Seven of these patients developed lymphomas, and in some of them clonal blood rearrangements preceded diagnosis of lymphoma with identical clonality by 2 years.

**Conclusions:** NBS patients often show clonal TCR and/or BCR gene rearrangements in PBMC. However, the clinical significance of this phenomenon requires further investigation.

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#### P04.170

#### Analysis of the 3'-UTR region of COX-2 gene in NMSC patients after transplantation

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Near up to 40% of transplant recipients experience non-melanoma skin cancer (NMSC). Various risk factors, including immunosuppressive treatment, infection with papiloma virus, and exposure to UV radiation contribute to the enhanced cutaneous carcinogenesis in these patients. UV radiation can promote carcinogenesis by its ability to induce formation of prostaglandins, which may then function as tumor promoters, or may enhance initiation because of their ability to act as oxidants. Cyclooxygenase-2 (COX-2) is the key enzyme in the production of prostaglandins. High levels of COX-2 has been reported in various epithelial cancer, including those of the skin. Expression of COX-2 are in part regulated by the 3'UTR which modulates the stability of the transcript. One polymorphism (8473T>C) in the 3' UTR region has been reported in association with breast and lung cancer risks.

This study was designed to investigate if polymorphism 8473T>C or other polymorphisms in the 3'UTR region can contribute to NMSC after transplantation. Genotyping of polymorphism 8473T>C in 150 patients and 180 controls demonstrated no significant differences, also stratifying by kind of tumor ( $p$  value>0.2). Moreover no appreciable difference were noted in functional analysis to test influence of C or T variant. Screening for new polymorphisms was performed in 30 NMSC and 30

controls, by heteroduplex analysis. We analyzed sequences containing binding sites for stabilization elements and putative sequences of microRNAs binding sites. One polymorphism was found (rs4648290) which is located in a microRNA binding site. Genotyping of this variant is ongoing and functional effects will be analyzed.

#### P04.171

#### Copy Number Alterations of HER2 and EGFR Genes in Non-Small Cell Lung Cancer (NSCLC)

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**PURPOSE:** Epidermal Growth Factor Receptor (EGFR) and HER2 are the members of EGFR family. They are transmembrane proteins with tyrosine kinase activity. EGFR and HER2 amplification and/or overexpression have been reported in several types of cancer. The study was performed to correlate copy number variations of HER2 and EGFR genes with histopathological features of NSCLC samples. MATERIALS-METHODS: Copy number status of the genes was assessed using FISH in 100 archival materials. RESULTS: 75 % of the samples successfully analysed by FISH. HER2 amplification was only detected in high grade tumors whereas EGFR amplification was significantly seen in high grade (16/75) tumors but also less frequently detected in low-grade samples. Both of the gene amplifications were detected in histologically proved squamous cell and adenocarcinoma tumors in about equal frequencies. Amplification of EGFR also correlated with smoking. CONCLUSION: The amplification of EGFR and HER2 in NCLSC patients may play important role in the pathogenesis of lung carcinoma.

#### P04.172

#### Studying Amplification Status of HER2/NEU and EGFR Oncogenes in Non-Small Cell Lung Cancers by Real-Time PCR Technique

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**AIM:** Family members of Epidermal growth factor receptor, EGFR and Her2/neu oncogenes, code transmembrane proteins which exhibit tyrosine kinase activity. The family members play major roles in signal transducing pathways. Amplification /overexpression of these protooncogenes have been identified in various cancer types including NSCLC. The study was performed to correlate copy number variations of HER2 and EGFR genes with histopathological features of NSCLC samples, smoking status and family histories of the cases. MATERIALS-METHODS: DNAs were extracted from formalin-fixed, paraffin-embedded sections obtained from 100 patients with NSCLC and amplification levels of HER2 and EGFR were assessed by real-time PCR. RESULTS: The HER2/neu and EGFR gene amplifications were measured quantitatively in 18% and 26% of tumors, respectively. HER2 and EGFR amplifications were significantly detected in high grade tumors and HER2 gene amplification was only seen in tumors of smoking cases. Amplification of EGFR also correlated with smoking. CONCLUSION: The amplification of EGFR and HER2 in NCLSC patients may play important role in the pathogenesis of lung carcinoma. Real-time PCR is easy to operate and deserves widespread application for detection of HER2 and EGFR gene copy number variations.

#### P04.173

#### Participation of OCT3/4 and β-Catenin during dysgenetic gonadal malignant transformation

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Gonadoblastoma (GB) is an *in situ* germ cell tumor, 30% of patients GB develops in dysgerminoma/seminoma. GB almost exclusively affects a subset of patients with Disorders of sex development (DSD), in which tumor development is associated with the presence of an extra Y-chromosome (either normal or abnormal), or with molecular

evidence for Y-derived sequences. GB in dysgenetic gonads and its counterpart, carcinoma *in situ* (CIS) in well-differentiated testicular tissue, may be the earliest stages in the development of malignant germ cell tumors (GCTs). Although the mechanisms that trigger neoplastic progression are still unknown, several pathways have been proposed. Abnormal OCT3/4 expression is the most robust risk factor for malignant transformation. This transcription factor regulates the pluripotency of embryonic stem cells and is necessary for the survival and migration of primordial germ cells and has been associated with the germ cell neoplastic process. OCT3/4 and β-catenin might both be involved in the same oncogenic pathway, both genes are master regulators of cell differentiation and overexpression of either gene may result in cancer development. The mechanism by which β-catenin participates in GB transformation is not completely clear. In an effort to elucidate the participation of β-catenin and E-cadherin, as well as OCT3/4, in the GB oncogenic pathways, we analyzed those molecules in seven patients with mixed gonadal dysgenesis and GB. Proposing that the proliferation of immature germ cells in GB may be due to an interaction between OCT3/4 and accumulated β-catenin in the nuclei of the immature germ cells.

#### P04.174

#### An inherited mitochondrial DNA disruptive mutation preferentially selected in oncocytic tumor cells

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Oncocytic tumors are characterized by cells with aberrant mitochondrial hyperplasia. Somatic mutations in mitochondrial genome (mtDNA) affecting respiratory chain complex I subunits have been previously reported in this type of neoplasia. We report the first case of inherited frameshift complex I mutation in the ND5 gene associated with a specific tumor phenotype.

The mtDNA was sequenced in microdissected areas from an oncocytic nasopharynx tumor and in different non-neoplastic tissues from the patient and two of his siblings in order to confirm inheritance of the mutation. Immunohistochemical analysis for complex I subunits was performed on tumor tissue to study protein expression and results confirmed by western blot. Quantification of the mutation load in mitochondria and of the total mtDNA copy number in the tissues analyzed for the patient and his siblings was performed. The oncocytoma harbors a frameshift homoplasmic (all mtDNA copies mutated) ND5 mutation which correlated with lack of expression of another mitochondrial coded complex I subunit (ND6). Conversely, oncocytic cells expressing ND6 show heteroplasmy for the ND5 mutation and a de novo homoplasmic pathogenic ND1 mutation. Such cells also present a lower degree of mitochondrial hyperplasia as shown by mtDNA copy number. The ND5 mutation is heteroplasmic in all normal tissues of the patient and his siblings indicating a shift to homoplasmy only in the tumor.

Since shift to homoplasmy of ND1 and ND5 mutations occurs exclusively in tumor cells, we conclude that complex I mutations may have a selective advantage and accompany oncocytic transformation.

#### P04.175

#### Functional gene polymorphisms of 16 factors that influence risk for oral cancer: A multivariate regression analysis of their effect

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**Introduction:** Functional DNA polymorphisms in genes of factors related to angiogenesis, inflammation and thrombosis have been associated with increased predisposition for oral squamous cell carcinoma (OSCC) by genetic association studies performed by our group and others. This study examined the possible combinatory effect of 16

such gene polymorphisms in predicting the occurrence of OSCC in Europeans.

**Methods:** 330 Greeks and Germans were studied, consisting of 162 OSCC cases and 168 healthy controls of comparable age, gender, and ethnicity. A series of multivariate logistic 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 studied polymorphisms included *IL-1b(+3953C/T)*, *IL-4(-590C/T)*, *IL-6(-174G/C)*, *IL-8(-251A/T)*, *IL-10(-1082A/G)*, *TNF- $\alpha$ (-308G/A)* and *TNF- $\beta$ (+252G/A)*, *MMP-1(-1607 1G/2G)*, *MMP-3(-1171 5A/6A)*, *MMP-9(-1562C/T)*, *TIMP-2(-418C/G)*, *VEGF(+936C/T)*, *GPI- $\alpha$ (+807C/T)*, *PAI-1(4G/5G)*, *ACE*(intron 16D/I) and *TAFI(+325C/T)*.

**Results:** The contribution of TNF- $\alpha$  and IL-6 polymorphisms was consistent and robust in almost all models constructed. Furthermore, when the mode of inheritance of each variant allele was taken into account in a multivariate logistic regression model, five polymorphisms emerged as primary predictors for all OSCC stages: *TIMP-2*(OR=26.33; 95%CI=12.39-55.95), *TNF- $\alpha$* (OR=15.27; 95%CI=7.30-31.96), *IL-6*(OR=8.33; 95%CI=3.95-17.58), *IL-8*(OR=3.54; 95%CI=1.69-7.43) and *IL-10*(OR=2.65; 95%CI=1.28-5.46).

**Conclusions:** The highly significant contribution of 5 out of 16 studied factors in the occurrence of OSCC was revealed. Based on these findings and previous reports, possible interactions of the implicated factors leading to OSCC development, as well as an algorithm of risk estimation will be discussed.

#### P04.176

#### P53 and bcl-2 in the pathogenesis of oral squamous cell carcinoma

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The imbalance between the rate of cell proliferation and apoptosis in tumour tissue is due to numerous genetic and epigenetic factors. Two of the most important cancer genes, contributing to pathogenesis of oral squamous cell carcinoma (OSCC) are p53 and bcl-2. Mutated p53 gene is associated with the inability of the cell to repair DNA lesions or to induce apoptosis. Consequently, increased expression of the antiapoptotic marker bcl-2 in tumour cells is expected. Based on this regulation pathway, in the present study we analyzed the association between the presence of mutated p53 gene and expression of bcl-2 protein. Mutational analysis of exons 5-8 of p53 was done by SSCP followed by sequencing. Expression of bcl-2 was analyzed by immunohistochemistry. According to results of mutational and immunohistochemical analyses, performed on 28 specimens of OSCCs, 60% of samples showed simultaneously the presence of mutated p53 gene and expressed bcl-2 oncogene. Independently of p53 status, the expression of bcl-2 was detected in 68% cases. Both analyzed markers showed an increased rate of mutation/expression in tumors of higher clinical stages.

The positive association between changes in p53 and bcl-2, point at deep deregulation of apoptosis which is in agreement with the increased proliferative potential of OSCCs.

Routine analysis of these markers in tumour samples obtained after surgery might be valuable in the assessment of tumour aggressiveness.

#### P04.177

#### Intratumoral patterns of clonal evolution in Ductal Pancreatic Adenocarcinoma by multicolour interphase fluorescence *in situ* hybridization (iFISH).

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Introduction. Pancreatic ductal adenocarcinoma (PDAC) is a fatal disease with an almost uniform 5-year mortality rates. Understanding the molecular mechanisms involved in tumor development and progression are a prerequisite to improve early diagnosis and therapy. How-

ever no study has been reported so far in which the clonal evolution of tumor cells has been systematically analyzed at the intratumoral cell level.

**Materials and methods.** Chromosomal abnormalities for 46 chromosomal regions distributed across the most frequently altered chromosomes were studied in 29 PDAC patients using multicolor interphase FISH techniques.

**Results.** All cases displayed numerical/structural abnormalities for at least one of the 24 chromosomes analyzed. Chromosome 17 was the most frequently altered (27/29 cases), followed by chromosomes 18 (23/29), 8 and 9 (24/29), and chromosome Y (17/21); in contrast, chromosome 22 was the less frequently altered. Of note, monosomy 9/9p+ was positively associated with 17p and 18q deletions. Overall, only 3 (7%) of the 29 cases analyzed had a single tumor cell clone while most cases (93%) displayed two or more clones. The most frequent ancestral tumor cell clones were characterized by deletion of 9p, 17p, 18q and Y nulism.

**Conclusions.** Our results show that, as in other tumors, progression of PDAC is a multi-step process in which neoplastic cells develop genome-wide instability evidenced at the intratumoral cell level leading to the emergence of multiple clonal populations with different chromosome abnormalities within the same tumor. This study also provides evidence about different patterns of intratumoral clonal evolution which had not been previously reported.

#### P04.178

#### Genetic variation and haplotype structures of the gene encoding human thymidylate synthase, a pharmacogenetic marker in fluoropyrimidine-treated cancer patients

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Thymidylate synthase (TS) activity is an important determinant of response to chemotherapy with fluoropyrimidine prodrugs and its expression is largely determined by polymorphic features in the 5' UTR region of the TS gene that can modify the number of the functional USF protein binding sites. DNA was extracted from peripheral blood from 587 caucasian cancer patients. Genotyping was performed by PCR amplification of the repeat region followed by automated sequencing

**Results:**

# Functional USF binding sites	Genotypes*	Colorectal cancer N (%)	Breast cancer N (%)
1	2RGC/2RCC	0 (0.0)	1 (0.6)
	2RCC/3RGCC	3 (0.7)	0 (0.0)
	2RGC/2RGC	75 (17.8)	40 (24.1)
	3RGCC/3RGCC	44 (10.6)	13 (7.8)
	2RGC/3RGCC	103 (24.5)	45 (27.1)
	2RCC/3RGCC	2 (0.5)	0 (0.0)
	2RGC/3RGCC+ins6pb	4 (1.0)	1 (0.6)
	3RGCC/3RGCC+ins6bp	3 (0.7)	2 (1.2)
2	3RGCC/3RGCC	75 (17.8)	25 (15.1)
	2RGC/3RGCC	73 (17.3)	27 (16.3)
	<b>2RGC+ins4bp/3RGCC</b>	1 (0.2)	0 (0.0)
	3RGCC/3RGCC+ins6bp	3 (0.7)	0 (0.0)
3	3RGCC/3RGCC	32 (7.6)	12 (7.2)
	2RGG/3RGCC	1 (0.2)	0 (0.0)
	2RGC/4RGCC	1 (0.2)	0 (0.0)
	3RGCC/4RGCC	1 (0.2)	0 (0.0)
Total N: 421			Total N: 166

In bold: novel allele. \*According to Lincz et al. Int. J. Cancer 2007; 120:1930-34.

We define the genotypes of the 5'UTR region of the TS gene in a large cohort of caucasian cancer patients and we determine the number of functional USF binding sites that can be used as a pharmacogenetic marker in patients treated with fluoropyrimidines. The recently described 2RCC allele was also found in 6 patients, in all cases in heterozygous form. We identify a novel allele containing a 4bp insertion (cgcc) in the inverted repeat sequence located 2bp prior to the ATG start codon.

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**P04.179****Molecular genetic analysis of apparently sporadic pheochromocytomas and paraganglioma in Czech patients****Z. Musil<sup>1</sup>, J. Vesela<sup>1</sup>, A. Krepelova<sup>1,2</sup>, A. Panczak<sup>1</sup>, J. Widimsky<sup>3</sup>, T. Zelinka<sup>3</sup>, A.****Puchmajerova<sup>2</sup>, M. Simandlova<sup>2</sup>, Z. Frynak<sup>4</sup>, J. Vaclavik<sup>4</sup>, M. Kohoutova<sup>1</sup>****<sup>1</sup>Institute of Biology and Medical Genetics of the First Faculty of Medicine, Prague, Czech Republic, <sup>2</sup>Institute of Biology and Medical Genetics, 2nd Faculty of Medicine and Teaching Hospital at Motol, Prague, Czech Republic, <sup>3</sup>3rd Medical Department - Clinical Department of Endocrinology and Metabolism, 1st Faculty of Medicine, Prague, Czech Republic, <sup>4</sup>3rd Clinical Department, Faculty of Medicine and Faculty Hospital, Palacky University, Olomouc, Czech Republic.**

Pheochromocytoma is a tumor arising in adrenal or extra-adrenal sites and occurs as a sporadic form or, less frequently, in familial setting as a part of inherited syndromes. Paraganglioma of the head and neck occurs mostly sporadically and also in syndromic or non-syndromic familial settings. To date, four susceptibility genes for pheochromocytoma have been reported that included *RET* proto-oncogene, *VHL* tumor suppressor gene, and recently identified genes *SDHB* and *SDHD* for succinate dehydrogenase subunit B and D, respectively. Mutations in these genes can predispose an individual to pheochromocytoma and/or paraganglioma. All these genes were analyzed to investigate possible genetic cause of pheochromocytoma and paraganglioma in population of Czech patients. Among 147 patients two novel germline (missense) mutations were found in the *VHL* gene. Further, one novel coding single nucleotide polymorphism and one inversion, both in *SDHB* gene, were detected. In one case of isolated pediatric onset of neuroblastoma and paraganglioma we detected a novel splice site mutation in *SDHB* gene. In addition, in one examined patient with paraganglioma we detected mutation of the start codon in *SDHD* gene.

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**P04.180****Carriers of germline c.1710+1355G>C substitution in the *PML* gene are at an increased risk of gastrointestinal cancer****P. Plevova<sup>1,2</sup>, S. Walczyskova<sup>1</sup>, I. Jeziskova<sup>1</sup>, A. Krepelova<sup>3</sup>, K. Langova<sup>4</sup>, E. Silhanova<sup>1</sup>,****<sup>1</sup>Department of Medical Genetics, Faculty Hospital, Ostrava, Czech Republic,****<sup>2</sup>Medical Faculty, Palacky University, Olomouc, Czech Republic, <sup>3</sup>3. Institute of Genetics, 2nd Medical Faculty, Charles University, Prague, Czech Republic,****<sup>4</sup>Institute of Biophysics, Palacky University, Olomouc, Czech Republic.**

Introduction: *PML* (promyelocytic leukemia) is a tumor suppressor gene. We studied occurrence of c.1710+1355G>C (p.A570+232>P570+232) substitution in alternatively spliced exon 7b of the *PML* gene in cancer patients.

Patients and methods: Sporadic and hereditary breast, colon and stomach cancer and multiple colon polyposis patients were included into the study. The control group included 100 non-cancer patients. The c.1710+1355G>C substitution was analyzed in genomic DNA using restriction analysis. Its occurrence in each cancer versus control groups was correlated using chi-square test.

Results: c.1710+1355G>C was found in 7 of 64 (11%) non-*BRCA1/2* breast cancer ( $p=0.683$ ), none of 24 *BRCA1/2*-associated breast cancer ( $p=0.276$ ), 15 of 57 (26%) non-HNPCC colon cancer or multiple polyposis ( $p=0.003$ ), none of 6 HNPCC-associated colon cancer ( $p=0.988$ , chi-square test with Yates correction), 2 of 3 stomach cancer patients ( $p=0.025$ , chi-square test with Yates correction) and 9 of 100 (9%) controls.

Conclusion: Our results suggest that germline carriers of the c.1710+1355G>C substitution in alternatively spliced exon 7b of the *PML* gene might be at an increased risk of gastrointestinal cancer or polyposis.

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**P04.181****The 8q24 rs10505470 variant is not associated with prostate cancer risk in patients from the Republic of Macedonia****N. Matevska<sup>1</sup>, D. Petrovski<sup>2</sup>, S. Dzikova<sup>2</sup>, S. Banev<sup>2</sup>, V. Georgiev<sup>2</sup>, A. Sikole<sup>2</sup>, A. J. Dimovski<sup>1</sup>,****<sup>1</sup>Faculty of Pharmacy, Skopje, The former Yugoslav Republic of Macedonia,****<sup>2</sup>Faculty of Medicine, Skopje, The former Yugoslav Republic of Macedonia.**

Recent compelling evidence demonstrates chromosome 8q24 as a prostate cancer (PC) susceptibility locus. Multiple variants within three adjacent regions at 8q24 have been identified to impact the risk of PC. Most commonly assessed variants are rs1447295 (region1), rs16901979 (region2) and rs6983267 (region3). Although regions 1 and 3 are close together, they are separated by a recombination hot-spot among individuals of European ancestry. Hence, all three neighboring regions seem to contribute independently to the PC risk, and the combined effects of SNPs across regions follow a multiplicative model. In order to examine the association between the PC risk and all three regions of 8q24 we designed a case-control study of randomly selected PC patients and controls without history of any malignant disease. Herein, we present the results of the association of PC risk and rs10505470 variant which is known to be in strong LD with rs6983267 in region 3. The rs10505470 genotypes were determined using custom designed TaqMan SNP genotyping assay on a Real-time PCR analyzer (MxPro 3005P). We did not observe a difference in overall allelic frequencies and genotype distribution {(A allele 0.518 for patients; 0.516 for controls); (AA 28.24%, AG 47.06%, GG 24.71% for patients; AA 27.60%, AG 47.92%, GG 24.48% for controls)}. Furthermore there was no significant difference after stratification of patients in subgroups according to age and Gleason score. Our findings led us to conclude that rs10505470 variant is not implicated with PC risk, time of onset or PC aggressiveness in Macedonian population.

**P04.182****Alpha-methylacyl-CoA racemase, a novel specific biomarker for prostate cancer****R. O. Dumache<sup>1</sup>, B. G. Bumbacila<sup>1</sup>, A. Anghel<sup>1</sup>, F. Miclea<sup>2</sup>, M. Puiu<sup>3</sup>,****<sup>1</sup>Victor Babes' University of Medicine and Pharmacy, Faculty of Medicine,****Biochemistry Department, Timisoara, Romania, <sup>2</sup>Victor Babes' University of****Medicine and Pharmacy, Faculty of Medicine, Urology Department, Timisoara,****Romania, <sup>3</sup>Victor Babes' University of Medicine and Pharmacy, Faculty of****Medicine, Medical Genetics Department, Timisoara, Romania.**

Alpha-methylacyl-CoA racemase, abbreviated in data bases as AM-ACR or P504S, is a 382 amino acid protein that plays a critical role in peroxisomal and mitochondrial beta oxidation of branched chain fatty acid and bile acid intermediates, dihydroxycholestanic acid and trihydroxycholestanic acid, molecules. Specifically, it catalyses the conversion of (*R*)  $\alpha$ -methyl branched chain fatty acyl CoAs to their (*S*) stereoisomers, because only stereoisomers with the 2-methyl group in the (*S*) position can be metabolized by peroxisomal oxidases, the first enzymes in the  $\beta$  oxidation pathway. It is well known that the main sources of branched chain fatty acids are the dairy products and red meat pork and beef, the consumption of which has been associated with an increased risk for prostate cancer in men, in multiple studies. We evaluated the AMACR gene expression in 32 patients aged 55 to 80, with total PSA values in a range of 2 to 40 ng/ml. 26 patients were diagnosed positive for prostate intraepithelial carcinoma and they had PSA values greater than 11 ng/ml. In these 26 men, we found by using PCR and micro array techniques on needle biopsies that they had an over expressed gene for AMACR, too. The other 6 patients with total PSA values smaller than 11 ng/ml, had normal expression of the AMACR gene.

According to our results, we propose the use of AMACR as an important marker of prostate cancer in Urology Clinics beside PSA and morphopathological diagnostic.

**P04.183****CAG repeat number in androgen receptor gene and prostate cancer****S. Trivodalieva<sup>1</sup>, N. Matevska<sup>2</sup>, A. Dimovski<sup>2</sup>, G. D. Efremov<sup>1</sup>, D. Plaseska-Karanfilska<sup>1</sup>,****<sup>1</sup>Macedonian Academy of Sciences and Arts, Research Centre for Genetic Engineering and Biotechnology, Skopje, The former Yugoslav Republic of Macedonia, <sup>2</sup>Faculty of Pharmacy, Farmacogenetic laboratory, Skopje, The former Yugoslav Republic of Macedonia.**

Prostate cancer (PC) is the second leading cause of cancer deaths among men. The effects of androgens on prostatic tissue are mediated by the androgen receptor (AR) through the androgen receptor-androgen complex. The 5' end of exon 1 of the AR gene includes a polymorphic CAG triplet repeat that varies in number between 10 to

36 in the normal population. The length of the CAG repeats is inversely related to the transactivation function of the AR gene. There is controversy over an association between short CAG repeat numbers in the AR gene and prostate cancer. The aim of this study was to evaluate the possible effect of short CAG repeats in the AR on prostate cancer risk in Macedonian males. We studied 66 PC patients, 46 patients with benign prostatic hyperplasia (BPH), 104 male patients with colon cancer (CC) and 152 males from the general population. The CAG repeat number was determined by fluorescent PCR amplification of exon 1 of AR gene followed by capillary electrophoresis on ABI PRISM 310 Genetic Analyzer. The mean CAG repeat length among PC patients was significantly lower ( $21.4 \pm 2.6$ ) in comparison to the BPH patients ( $22.7 \pm 2.2$ ;  $p=0.007$ ), CC patients ( $22.6 \pm 3.1$ ;  $p=0.008$ ) and males from general population ( $22.3 \pm 2.8$ ;  $p=0.029$ ). We found a significantly higher percentage of short CAG repeats ( $\leq 21$ ) in PC patients (56.1%) than in BPH patients (21.7%;  $p = 0.0002$ ). Our results suggest that short CAG repeats are associated with an increased prostate cancer risk in Macedonian men.

#### P04.184

#### The Galician splicing mutation p.R71G in the *BRCA1* gene is not found at an increased frequency in Galician prostate cancer patients

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The Breast Cancer Linkage Consortium and other family-based ascertainment have suggested that male carriers of *BRCA* mutations are at increased risk of prostate cancer. However, several series looking at the frequency of *BRCA* mutations in unselected patients with prostate cancer have not confirmed this finding. In the Galician population, one splicing founder mutation p.R71G in the *BRCA1* gene is present in more than 50% of the breast/ovarian cancer families with mutations. To assess the contribution of p.R71G to prostate cancer morbidity we conducted a case-control study in prostate cancer patients. Blood specimens from 257 unselected Galician men with prostate cancer were screened for the presence of the common Galician founder mutation in *BRCA1*. As a control group 250 Galician male volunteers without prostate cancer were genotyped. We found that mutation p.R71G was completely absent in our cohort. Therefore, our result suggests that the contribution of *BRCA1* germline mutations in prostate cancer morbidity is probably negligible.

#### P04.185

#### Study of *GSTM1*, *GSTT1* polymorphisms and CAG microsatellite repeat length of the androgen receptor gene in Iranian prostate cancer patients

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Androgens which act through androgen receptors (ARs) are required for growth and normal prostate function. AR gene contains CAG repeats and it has been hypothesized that, shorter alleles of the CAG repeat are associated with an increased risk of prostate cancer. Ethnic variation in CAG repeat length may contribute to variable prostate cancer risks in different populations. Genetic polymorphism of metabolic enzymes such as Glutathione S-transferases is involved in metabolism of numerous potential prostate carcinogens. The frequencies of homozygous carriers of *GSTT1*, *GSTM1* deletions vary significantly within ethnic groups and may be contributed to prostate cancer risk. To evaluate whether these polymorphisms are associated with an increased risk in Iranian prostate cancer patients, DNA from 110 pathologically-confirmed prostate cancer patients, 96 age-matched men with BPH individuals were extracted and amplified by polymerase chain reaction

(PCR). PCR products were examined by electrophoresis, sequencing. Statistical analysis of results was carried out using t-test and chi<sup>2</sup> methods. The mean number of CAG repeat in prostate cancer patients was significantly smaller than BPH groups (19.9 vs. 21.9  $P < 0.0001$ ). The frequencies of *GSTT1* and *GSTM1* positive genotypes were higher in patients (65.5%, 54.9%) than BPH group (59.1, 52.3). Our results showed that CAG repeat polymorphism in AR gene is significantly associated with higher prostate cancer risk, suggesting that it may act as a risk modifier to cancer patients in Iranian population. *GSTT1* and *GSTM1* positive genotypes also may be contributed to prostate cancer susceptibility in Iranian patients.

#### P04.186

#### TMPRSS2/ERG fusion gene is a novel hormone refractory marker of prostate cancer

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Recurrent gene fusions involving oncogenic ETS transcription factors (including ERG, ETV1, and ETV4) have been identified in a large fraction of prostate cancers. The most common fusions contain the 5' untranslated region of TMPRSS2 fused to ERG. TMPRSS2-ERG gene fusion leading to the androgenic induction of the ERG proto-oncogene expression is a highly prevalent oncogenic alteration in prostate tumor cells. To assess the role of TMPRSS2-ERG alteration in prostate cancer onset and/or progression, we have evaluated the status of fusion transcripts in 72 whole-mount prostatectomy specimens of patients with pT1-T4 prostate cancer stage. We tested for the presence of TMPRSS2:ERG, TMPRSS2:ETV1 and TMPRSS2:ETV4 gene fusion product, using RT-PCR and direct sequencing. Overall, 36 of 72 (50%) patients exhibited TMPRSS2-ERG fusion transcripts in prostate cancer samples. There are no TMPRSS2:ETV1 and TMPRSS2:ETV4 fusion transcripts in adenocarcinoma specimens. TMPRSS2-ERG expression was correlated with prostate cancer stage. We observed significantly difference between hormone ablation therapy patients group and group without hormone therapy ( $p=0.02$ ). Patients without TMPRSS2-ERG expression are shown no response to hormone treatment.

TMPRSS2-ERG is the most common genetic aberration so far described in human malignancies. Furthermore, its clinical application as a hormone resistant biomarker is promising perspective for prostate cancer therapy scheming.

#### P04.187

#### Mitochondrial implication in Prostate Cancer: Mitochondrial gene expression

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Prostate cancer (PCa) is one of the most common cancers among male population in the world, being responsible for 6% of all deaths by cancer in males. In spite of its relevance and the progressive increase in the number of cases detected, the mechanisms that account for its genesis and development are unknown. Genomic studies have suggested that genes from mitochondrial respiratory chain, Krebs cycle and the glutathion reductase anti-oxidant system may be involved in PCa. In order to broadening our knowledge on the mitochondrial role in PCa carcinogenesis and progression, we have analyzed in paired samples (normal-tumor) from PCa patients, the presence of mutations in mitochondrial genome (by direct sequencing); mtDNA amount (by quantitative PCR); and mRNA expression of genes with mitochondrial functions encoded either by mitochondrial genome (*MT-ND2*, *MT-ND4*, *MT-ND6*, *MT-CYB*, *12S*, *16S*, *MT-CO2*, *MT-ATP6*) or by nuclear genome (*COX11*, glutathion reductase/GSR, citrate synthase/CS, aconitase/ACO2).

We have observed that in spite of the presence of mutations in mtDNA,

there are no changes in the amount of mtDNA. We have also observed a significance decrease in the expression of mitochondria-encoded genes *MT-12S*, *MT-CO2* and *MT-ATP6*.

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#### P04.188

##### **Identification of novel molecular targets for arresting prostate cancer cell proliferation.**

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Retroelements (RE) represent a significant portion of the human genome (45 %) and play a key role in the regulation of gene expression in mammals. RE can act as insertional mutagens altering the coding integrity of genes and, particularly the gene coding for reverse transcriptase (RT) is typically expressed at high level in transformed cells and tumor.

A large body of published data supports the conclusion that retrotransposons are biologically active elements and indicates that retrotransposition is an ongoing process in mammalian genomes and can trigger the onset of several pathologies including cancer. Active LINE-1 transcripts have been detected in murine embryonal carcinoma cells and in human testicular cancers, while only basal levels of RT activity have been revealed in terminally differentiated cells and tissues.

Studies developed in our research groups over the last years have shown that the commercially available RT inhibitor, Abacavir, widely used in AIDS therapy, is able to modulate cell growth and differentiation in prostate cancer cell line (PC3).

ABC, respectively at 10 and 100 µg/ml, is able to reduce proliferation, migration and invasion. We studied genome wide expression profile on PC3 cells treated with ABC and we identified genes involved in RNA regulation and expression. Further immuno-fluorescence experiments revealed a critical involvement of nucleolus as target of ABC action.

Finally, we discuss new approaches to treatment, including recently discovered molecular targets that might provide more effective treatment strategies with the potential for less toxicity.

#### P04.189

##### **PTCH1 gene polymorphisms and risk of non melanoma skin cancer after organ transplantation**

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Organ transplant recipients (OTR) are at higher risk of non melanoma skin cancer (NMSC), particularly basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Risk factors for NMSC in OTR include skin type, ultraviolet light exposure, immunosuppression, human Papilloma virus infections, and genetic susceptibility.

PTCH1 is a negative regulator of the Hedgehog pathway, that provide mitogenic signals to basal cells in skin. PTCH1 gene mutations are responsible for nevoid basal cell carcinoma syndrome (NBCCS or Gorlin syndrome), and also occur in sporadic forms of BCC. Associations have been demonstrated between PTCH1 polymorphisms and BCC susceptibility in non transplanted recipients.

This study was designed to investigate if known polymorphisms of PTCH1 gene contribute, individually or as haplotypes, to NMSC risk after transplantation, and to identify novel genetic polymorphism in the proximal 5' regulatory region of the gene.

We analyzed the frequencies of three PTCH1 gene SNPs (rs2297086, rs2066836, rs357564) in 273 Northern Italian OTR patients (120 cases and 153 controls).

Single locus and haplotype frequency analysis showed no significant associations.

Screening for polymorphisms was performed by heteroduplex analysis in 30 cases and 30 matched controls.

Two polymorphisms, -198A>G and -195G>C, were identified in the 5' flanking region. Both variants were in linkage disequilibrium and showed a frequency of 0.5% (2/400) in 200 tested individuals.

These results seem to exclude an important role of the PTCH1 gene in NMSC susceptibility after organ transplantation. Further studies on a larger sample are warranted to confirm these findings.

#### P04.190

##### **Molecular genetic and clinical survey of Swiss PTEN hamartoma tumor syndrome patients**

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PTEN hamartoma tumor syndrome (PHTS) is a condition caused by germline mutations in the PTEN (phosphatase with tensin homology) tumor suppressor gene which encodes a phosphatase with lipid and protein specificity, a negative regulator of the phosphoinositol 3-kinase (PI3K) pathway. PHTS includes Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRSS), Proteus syndrome (PS), and Proteus-like syndrome, the first two being the most prevalent conditions. Here we present the results of a molecular genetic and clinical survey on 12 Swiss PHTS patients (10 CS, 2 BRSS) and their relatives referred during the past 4 years. All patients were subjected to DNA sequencing of the coding (incl. exon/intron boundaries) and the promoter region, and to gene dosage analysis. In the coding region of PTEN, heterozygous germline mutations were identified in five CS patients and one BRSS patient. The majority of the PTEN mutations were found to be either nonsense point mutations or frameshift deletions. In addition, one heterozygous splice site mutation and one missense mutation were observed. Based on these findings, the mutational spectrum and clinical manifestations in both, PTEN mutation carriers and those without detectable genetic alterations will be presented. In particular, the clinical overlap between CS and BRSS PTEN mutation carriers will be discussed.

#### P04.191

##### **Evaluation of exon 2 and exon 6 mutations of PTEN gene in patients with gastric cancer**

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Gastric cancer (GC) is still one of the most frequent causes of cancer-related deaths in all overworld. Molecular mechanism of GC has not been well described. In recent years, the pathogenesis of GC related to the basis of the molecular and genetic changes have been investigated in the different populations. These changes can be classified as activation of oncogenes, the inactivation of tumor suppressor genes, the reduction of cellular adhesion, the reactivation of telomerase and the presence of microsatellite instability. A novel tumor suppressor gene, PTEN (phosphatase and tensin homologue deleted on chromosome ten), has recently been described and mapped to chromosome band 10q23.3 region. In the present study, we evaluated the frequency of exon 2 and exon 6 mutations of PTEN gene in the normal (n=47) and tumor tissues (n=47) of the patients with GC, by using SSCP and sequencing techniques. The frequency of mutations in exon 2 and exon 6 of the PTEN gene in tumor and normal tissue DNAs were compared and the results were compared with the other results published for the other population in the literature.

#### P04.192

##### **High throughput, nanofluidic real-time PCR analysis of gene expression in tumor samples**

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Understanding biological complexity arising from patterns of gene expression and gene function requires accurate and precise measurement of RNA levels across large numbers of genes simultaneously. We demonstrate a novel highly parallel, nanofluidic system capable of performing 3072 real-time polymerase chain reactions (rt-PCR), based on Sybr Green detection, in a miniaturized through-hole array format.

The system has shown accuracy, precision and dynamic range in the thirty-three nanoliter reaction volumes identical to the same reactions performed in 100-fold larger volumes typical for rt-PCR in 384-well microplates. A 64-fold increase in analytical throughput relative to 384-well microplates simplified quantification of message RNA, resulting in unprecedented throughput and sensitivity suitable for detection of low abundance nucleic acids as well as low consumable costs. We show the utility of this system for studying two different areas of cancer biology. First, the expression of over 500 kinase genes was screened in three types of tumor tissues and the biological significance of regulated genes in regulating the cell cycle was confirmed. Second, we studied its utility for screening for DNA methylation as a biomarker for tumor detection.

#### P04.193

##### **Setting up of a questionnaire by the genetic counselor to optimize the collection of data during the first consultation of cancer genetics**

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Persons presenting a hereditary predisposition for cancer are addressed to a consultation of cancer genetics because of their personal and family history of cancers. During consultation a family pedigree is drawn, genetic counselor collects information to evaluate hereditary predisposition. All this information is difficult to obtain if the patients are not informed of the questions which will be asked to them during consultation.

The aim of this study is to optimize the collection of information using a questionnaire targeting essential data during this first consultation.

This questionnaire was addressed to all patients making an appointment for a first consultation. It gathers personal and medical information, in both parental branches over three generations. Questionnaires were assessed according to two criteria : their return and filling up rates.

The return rate is 82 % (110/134). The mean filling up rate is 74 % and for more than 86 % of questionnaires, the amount of information is very satisfactory (questionnaires supplemented in 60, 80 and 100 %).

The questionnaire has a double interest, for the patients and for the medical team. The patients collected a lot of information on their family before their consultation. For the medical team, data necessary for the realization of the family pedigree are gathered in a more precise and quicker manner.

Considering the good return and filling up rates of this questionnaire, we decided to insert it into the daily practice of our consultations.

#### P04.194

##### **Analysis of the molecular-genetic alterations in the genes VHL, RASSF1, and FHIT in clear cell renal carcinomas**

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Clear cell renal carcinoma accounts approximately 75% patients with kidney cancer and characterized by alterations of the genes *VHL*, *RASSF1*, *FHIT*. We have conducted the molecular-genetic study of mutations, loss of heterozygosity, methylation of the *VHL* gene as well as allelic deletions of the genes *RASSF1* and *FHIT* in 123 clear cell renal carcinomas for the development of renal cancer prognostic criteria. *VHL* mutations were detected by SSCP and subsequent sequencing; methylation was tested by methylsensitive polymerase chain reaction. Loss of heterozygosity was analyzed using STR-markers D3S1317 and D3S1038 (*VHL*), D3S1568 and D3S966 (*RASSF1*), D3S1234 and D3S1300 (*FHIT*). *VHL* somatic mutations were observed in 31.7% samples, 84.6% of them were identified for the first time. We have detected *VHL* allelic deletions in 27.9% informative cases, and aberrant methylation - in 14.6% heterogeneous tumor samples. *VHL* inactivating events were presented in 53.8% patients with stage I, and could be responding for early gene inactivation. *RASSF1* and *FHIT* allelic deletions were observed in 27.5% and 35.6% informative cases, correspondingly. Loss of heterozygosity of two or three analyzed genes in

primary tumor was associated with metastases in the regional lymph nodes and/or distant metastases ( $P = 0.036$ , OR = 1.49, 95% CI: 1.01-2.33). Results of this investigation could be used for selection of prognostic molecular markers of clear cell renal cancer.

#### P04.195

##### **Under-representation of the RET sequence variants G691S and S904S in patients with a common C618R RET proto-oncogene mutation**

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Medullary thyroid carcinoma (MTC) occurs in a sporadic or as an autosomal dominant hereditary form. Inherited forms of MTC are related to mutations in the RET proto-oncogene. Identification of polymorphic variants that increase susceptibility and variations in pathological phenotypes is a frequent question that is addressed in medical genetics. In various studies which resulted to conflicting results a number of polymorphisms within the RET proto-oncogene were proposed to act as low penetrance predisposing alleles. In the present study, in order to test for the contributory role of the polymorphisms G691S and S904S, in the development of MEN2A, we looked for an association of these RET haplotypes in hereditary MEN2A cases and in controls from individuals of Greek Cypriot origin.

Screening for the polymorphic variants G691S/S904S was performed in 226 Greek Cypriots who served as controls and in 6 unrelated Greek Cypriot patients diagnosed with MEN2A with the C618R mutation. In controls, the allelic frequencies of G691S/S904S polymorphisms were found in the heterozygous state in 102/226 (45 %) individuals, and in the homozygous state in 11/226 (~5 %). In all cases, these polymorphisms were in co-segregation. This allelic frequency for the polymorphisms G691S/S904S of the RET gene in the Greek Cypriot population is the highest reported so far among normal individuals. Moreover, only 1/6 (16.66 %) patients with MEN2A had the G691S/S904S polymorphisms in the heterozygous state. This under-representation of the G691S/S904S polymorphic variants in MEN2A patients, might suggest a possible synergistic and protective effect exercised by these polymorphisms.

#### P04.196

##### **Variants in the retinoblastoma gene: neutral polymorphism or pathogenic change?**

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Retinoblastoma is a malignant neoplasm of the developing retina, occurring mostly in early childhood. The birth prevalence is between 1 in 15.000 and 1 in 20.000. Hereditary (40% of cases) as well as non-hereditary (60% of cases) retinoblastoma (RB) result from inactivation of both alleles of the tumor-suppressor gene *RB1*, located on chromosome 13q14. In hereditary RB, the mutated gene is transmitted in an autosomal dominant way, usually with almost complete penetrance. We have performed DNA-mutation scanning (sequencing of exon 1, 15 and the *RB1*-promoter, DGGE analysis of the other exons and MLPA analysis for large deletions and duplications) in 411 RB-patients and found causative *RB1*-mutations in 173/197 (88%) bilateral/familial patients and 18/214 (8.4%) in unilateral non-familial cases. Most mutations are nonsense changes or small insertions or deletions causing frame-shifts. However, for some changes it is not immediately clear if they are pathogenic mutations or just neutral variants. These include missense changes, synonymous changes, small in-frame deletions and intronic variants at other sites than the invariable splice donor and acceptor sequences. These variants cause serious problems in genetic counselling of RB patients and their family. We will discuss several variants with unknown significance in the *RB1*-gene, 15 missense changes, 11 intronic variants, 2 synonymous changes en 1 in frame deletion, and illustrate ways to substantiate their pathogenicity.

**P04.197****Mutation in siRNA target sequence can impair RNAi mediated inhibition of E1A gene expression in HEK 293****H. Vosgha, M. Behmanesh, M. Sadeghizadeh;***Tarbiat Modares University, Tehran, Islamic Republic of Iran.*

RNA interference (RNAi) has emerged as an effective method for silencing gene expression in eukaryotic cells. It has tremendous potential as both a research tool and a therapeutic strategy. The key player in RNAi is small RNA (~ 22nt) termed siRNA. So in this report we used the E1A specific siRNA coding plasmid under U6 snRNA promoter to suppress E1A gene expression. Then these constructs were transfected into the HEK 293 cancerous and successfully transfected cells colonies were selected based on Neomycin antibiotic resistance. Changes in E1A gene expression were analysis using RT-PCR technique. Final findings showed no obvious difference in E1A gene expression level in suppression and control groups upon transfection with constructed plasmids. In order to deduce the rationale behind no suppression of the E1A gene expression, we analyzed the PCR amplified sequence of the siRNA target region of the E1A gene using sequencing technique. The findings illustrated certain mutations in this region. It has been established previously that in RNAi process, occurrence of any mutation in mRNA sequence of target gene at the siRNA binding site might cause impaired interference in gene expression. Therefore even a single mutation in mRNA sequence cause inhibition of interference.

**P04.198****Novel somatic mutations in the S100A2 gene in non-small cell lung carcinoma (NSCLC)****M. Strazisar, D. Glavac, T. Rott;***Faculty of Medicine, Ljubljana, Slovenia.*

Contrary to the recent hypothesis that S100A2 is a tumour suppressor, no somatic mutations have yet been identified. We therefore screened 90 non-small cell lung carcinoma (NSCLC) samples, initially for mutations in S100A2 and then also for mutations in P53 and K-RAS genes. Alterations were detected in 46.7 % of squamous lung cancer (SCC) samples, but we detected only one novel tumour specific mutation, Q23X in squamous carcinoma. We detected four polymorphisms, two of them published for the first time (144+109 C/G and 297+75A/G) and two already published: S62N, in coding region and related to squamous cell carcinoma, and 297+17T/C. In one tumour with the S62N polymorphism, P53 and K-RAS genes were also mutated, while two tumours with the Q23X mutation have a P53 but no K-RAS mutation. Expression profiles of hTERT and COX-2 revealed no significant correlation with tumours having also the S100A2 alterations. To the best of our knowledge, this is the first report describing alterations in the S100A2 gene proving the relation between polymorphic changes in predominantly squamous lung cancer (SCC).

**P04.199****Molecular cytogenetic characterization of paraffin-embedded salivary gland tumors by Comparative Genomic Hybridization****G. Floridia<sup>1</sup>, F. Censi<sup>1</sup>, M. Foschini<sup>2</sup>, V. Falbo<sup>1</sup>, D. Taruscio<sup>1</sup>;**<sup>1</sup>*Istituto Superiore di Sanità, Dept. Cell Biology and Neuroscience, Roma, Italy,*<sup>2</sup>*Section of Pathology, University of Bologna, Bellaria Hospital, Bologna, Italy.*

Salivary gland tumours (SGTs) are rare tumors of the neck and head with an overall incidence in the Western world of approximately 2.5-3/100.000/year. SGTs are remarkable for their histopathologic and biologic diversity; they include benign and malignant tumors of epithelial, mesenchymal and lymphoid origin. The study of molecular pathogenesis of SGTs is a challenging task because of the rarity and histopathological diversity of these malignancies. Comparative Genomic Hybridization metaphase-based was performed in 10 paraffine embedded Adenoid Cystic Carcinoma samples (ACC). Heterogeneity was detected and gains predominated over losses; no recurrent anomaly was observed. However 3q29, 5q35, 16q24 and 21q22 gains, detected in our study, have been reported and described in literature as ACC loci. The correlation of CGH results with clinical-pathological data and a comparison with literature data will be discussed.

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**P04.200****A case of synovial sarcoma of the pericardium diagnosed by FISH on FNA material****I. Trigo<sup>1</sup>, A. Hernandez<sup>2</sup>, M. Vargas<sup>1</sup>, M. Diaz<sup>2</sup>, J. Rios<sup>2</sup>, H. Galera-Ruiz<sup>2</sup>, R. Gonzalez-Campora<sup>2</sup>;**<sup>1</sup>*Unidad de Genética., Sevilla, Spain, <sup>2</sup>Dpt of Pathology, Sevilla, Spain.***Case report**

We present a case of 40 year-old woman, without any previous interesting medical history, who presented dyspnea, cough and fever over several weeks. Thoracic MR revealed a left posterolateral paracardial mass of possible pericardium origin. The cytologic study on material obtained by transesophageal FNA was performed and the tumour was surgically removed. Morphologic diagnosis was a malignant spindle cell tumour consistent with synovial sarcoma (immunohistochemistry profile: EMA, bcl-2,CD-99 and CK 19 positive. S-100, CD-34 and caldesmon negative). SYT gene rearrangement was confirmed by FISH techniques, verifying the localization at SSX2 gene.

The synovial sarcoma is an uncommon mesenchimal tumour with variable epithelial differentiation and represents 5% of cardiac sarcomas and less than 0.1 of all cardiac tumours. It is characterized by t (X;18)(p11;q11), which is present in its four histological types. The microscopic diagnosis is very difficult and the immunochemical techniques are helpful but not conclusive. Consequently other ancillary techniques, such as FISH analysis, are primordial to precise the definitive diagnosis of this entity characterized by the SYT gene rearrangement to SSX2 gene. In the literature review only 18 cases of heart synovial sarcoma have been described and only one of them (biphasic type) was diagnosed by FNA material without FISH contribution. To our knowledge no other FISH studies on cytopathologic material of synovial sarcoma of the pericardium have been reported in the literature.

**P04.201****Detection of human telomerase gene (*TERC*) amplification in cervical neoplasia: A retrospective study of 79 patients with normal smears or mild or moderate dyskaryosis****R. E. Crookes<sup>1</sup>, M. Dyson<sup>1</sup>, J. H. F. Smith<sup>2</sup>, E. Maltby<sup>1</sup>;**<sup>1</sup>*NHS Foundation Trust, Sheffield, United Kingdom, <sup>2</sup>Royal Hallamshire Hospital, Sheffield, United Kingdom.*

The inclusion of a genetic marker of disease progression for cervical carcinoma along side the histological assessment of Pap smear slides could dramatically reduce the number of diagnostic colposcopic procedures currently performed and increase the sensitivity and specificity of cervical screening programmes.

Recent CGH studies (Heselmeyer-Haddad et al., 2003) indicated the involvement of extra copies of 3q and hence the human telomerase gene (*TERC*) at 3q26, in invasive cervical carcinoma. Their retrospective study of 59 cervical smears showed that gains of *TERC* could predict progression from lower grade smear abnormality (mild or moderate dyskaryosis) to CIN3 and invasive carcinoma.

We present the results of retrospective study, using the same *TERC* probe kit, on a selection of 79 patients with negative, mild or moderate dyskaryosis that later progressed to CIN3/cervical cancer, or regressed to negative. In the cohort of patients classified as cytologically negative that subsequently developed CIN 3, 28.5% were positive for *TERC* gain. In the cohort of patients classified as mild or moderate that progressed to CIN3/cervical carcinoma, 60% and 82.4% respectively showed gains of *TERC*. In the cohort patients classed as negative, mild or moderate dyskaryosis that regressed or remained negative 30 out of 33 patients were negative for *TERC* gains. This concurs with previous studies which have proposed that the acquisition of *TERC* is an important event in the progression of cervical dysplasia to cervical cancer. The results demonstrate the potential use of *TERC* as an early prognostic detection of disease progression.

**P04.202****Effects of somatic mutations in TP53 on expression of genes involved in cell cycle arrest****D. Macic<sup>1</sup>, L. Kapur<sup>1</sup>, J. Ramic<sup>1</sup>, N. Lojo-Kadric<sup>1</sup>, N. Obradic<sup>2</sup>, T. Ceric<sup>2</sup>, S. Beslja<sup>2</sup>, K. Bajrovic<sup>1</sup>;**<sup>1</sup>*Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina, <sup>2</sup>Institute of Oncology, Sarajevo, Bosnia and Herzegovina.*

The TP53, tumor suppressor gene, is a critical regulator of cell division and its inactivation at the gene or protein level contributes to onco-

genesis and cancer progression. Mutations in TP53 are considered to represent the most common genetic alteration and occur in about 50% of human cancers. These mutations may damage the normal function of p53 as a transcription factor and the induction of repair or apoptosis may be diminished. Consequently, genetic alterations may accumulate in the cell.

Evidences from previous studies imply that there is a clear correlation between mutational status of TP53 and expression of genes down the p53-signaling pathway.

We examined the impact of mutations in DNA binding domain of p53 on expression of genes involved in cell cycle arrest, particularly CDK4 and its inhibitor CDKN2.

This study included biopsic samples from 80 breast and colon cancer affected subjects with different malignancy grades.

To test hypothesis that mutation in TP53 can influence expression of CDK4 and CDKN2, TP53 has been subjected to mutational analysis by RFLP followed by sequencing.

Expression of genes involved in cell cycle arrest was measured using SYBR-green based real-time PCR.

Mutations in TP53 were detected in 8% of the examined cases.

We compared expressions of CDK4 and CDKN2 in the samples that harbor a mutation to those without mutations.

Pathohistological findings obtained from clinic were correlated with molecular alterations.

#### P04.203

#### Association study of codon 10 polymorphism of the Transforming growth factor-beta 1 (TGF- $\beta$ 1) gene with prostate cancer and hyperplasia

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**Introduction:** Transforming growth factor-beta 1 (TGF- $\beta$ 1) has a multifactorial role in the development of cancer. Genetic polymorphisms in codons 10 of the TGF- $\beta$ 1 gene have been shown to be associated with the production of high or low TGF- $\beta$ 1 levels. The role of this polymorphism in development of prostate cancer and hyperplasia was investigated.

**Material and Methods:** Using ASO-PCR method, association between the T (Leu) to C (Pro) polymorphism at codon 10 of the TGF- $\beta$ 1 gene (TGFB1) and the risk of PCa or BPH in 100 controls were determined.

**Results:** Significant differences in the CC versus TT genotype distribution between PCa patients and male controls ( $P = 0.009$ ), and between BPH patients and male controls ( $P = 0.005$ ) were noticed. Males with the TT genotype had a 1.67-fold increased risk of PCa [95% confidence interval (95% CI) = 1.49-1.87,  $P = 0.009$ ] and TC /TT genotype, 1.14-fold increased risk of PCa [95% confidence interval (95% CI) = 1.02-1.26,  $P = 0.047$ ] and a 1.54-fold increased risk of BPH with the TT genotype (95% CI = 0.98-1.14,  $P = 0.005$ ) and 1.06-fold increased risk of BPH with the TT/TC genotype (95% CI = 0.98-1.14,  $P = 0.061$ ) compared with those with the CC genotype respectively.

**Conclusion:** Based on our findings, it was possible to conclude that the codon 10 polymorphism in TGFB1 may have a significant influence on the development of PCa and BPH and the T allele of TGFB1 gene has the dominant effect on development of PCa and BPH.

#### P04.204

#### Subtle microsatellite instability in pediatric gliomas as an indicator of type 1 Turcot syndrome

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Type 1 Turcot syndrome (TS1) is characterized by the association of early-onset glial and colorectal tumors and is caused by mutations of the mismatch repair genes. To determine the role of genetic predisposition in glial tumor development, we investigated the frequency of microsatellite instability (MSI) in a series of 38 pediatric gliomas using a panel of 5 quasimonomorphic mononucleotide markers. A pattern of "subtle" MSI for most tested markers was observed in 2 glioblastomas.

In both cases family history was indicative of TS1, as confirmed by the detection of mutations in the *PMS2* and *MLH1* genes. In one case, instability was revealed in diluted DNA samples. Comparison of the MSI patterns in normal (leukocyte and intestinal mucosa) and tumor (glioblastoma and colorectal cancer) samples from one TS1 family revealed that allelic size shifts were smaller in gliomas. Our results indicate that MSI analysis is an important tool to identify TS1-related pediatric gliomas, and that the pattern of microsatellite alterations in gliomas is less pronounced compared to colorectal cancer

#### P04.205

#### Analysis of the molecular genetic changes in uveal melanoma

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Uveal melanoma (UM) is the most common primary cancer of the eye and has a strong predilection for hematogenous metastasis, particularly to the liver. Investigation of the specific genetic mechanisms responsible for the malignant behavior of UM could play an important role for the development of new approaches to UM diagnosis and treatment. We investigated 105 UM for allelic losses at chromosomal regions 3p, 3q, 1p, 9p23, 10q23, 13q14, close or within some tumor suppressor genes (*VHL*, *FHIT*, *RASSF1A*, *CDKN2A*, *PTEN*, *RB1*). Moreover, we investigated the methylation status of the promoter regions of these genes.

45 UM (43%) had LOH at all informative loci of chromosome 3, indicating of monosomy 3. Methylation analysis discovered frequent methylation of *RASSF1A* (24 patients, 23%), located at 3p21.3 and inactivated in a large number of human cancers. Important to notice, methylation of *RASSF1A* was found predominantly in UM without monosomy 3 (20 patients). These findings could reinforce the role of *RASSF1A* in the pathogenesis of UM.

LOH at 1p was found in 28 samples mainly at all informative loci (5 MS markers, 1p31.3 to 1p36.3) without any relation to the monosomy 3. Five samples had partial allelic losses at 1p31.3 or 1p36.3. 1p contains wide range of TSGs, and for the moment we could not identify those who might be candidate genes for the UM. Additionally, we conclude that inactivation of the regulation pathway *CDKN2A*→*RB1* with promoter methylation or LOH is not the major mechanism of the pathogenesis in UM unlike cutaneous melanoma.

#### P04.206

#### VANGL1 effects cell invasion rather than cell motility

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Van Gogh like 1 (Vangl1) is a transmembrane protein on Wnt planar cell polarity pathway. It has an important role in planar cell polarity and convergent extension in embryonic development. In adults, it is expressed specifically in testis and ovaries as well as in brain and prostate. VANGL1 expression has been shown in several human cancer cell lines including hepatocellular carcinoma (HCC). Our aim in this study was to investigate the changes in the behaviour of the HCC cells whose VANGL1 gene was silenced by siRNA.

VANGL1 expression in HCC cell lines was shown by RT-PCR and real time PCR. The siRNA template which will transcribe the specific hairpin siRNA for VANGL1 gene was designed following the determination of the target sequence. The siRNA template was ligated to a siRNA expression vector and HepG2 cells were transfected. The colonies expressing the siRNA were detected by RT-PCR, quantitative RT-PCR and Western blotting. Motility and invasion of the cells were assessed by Boyden chamber assay while cell cycle analysis was performed by flow cytometry.

The motility of the cells was not effected with gene silencing while there was a three fold decrease in the invasion potential of the cells expressing the siRNA. The proliferation assay revealed no significant difference between the transfected cells and parental cells with regard to S phase cell ratio.

In conclusion, VANGL1 gene has an effect on cell invasion rather than cell motility. Further investigations are needed to understand the mechanisms of this effect.

**P04.207****Mutational spectrum of missense VHL gene mutations in Spain and their genotype-phenotype correlation**

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Von Hippel-Lindau (VHL) disease (MM#193300) is an inherited neoplastic disorder characterized by a predisposition to develop mainly retinal angiomas (RA), central nervous system hemangioblastomas (HB), clear cell renal carcinomas (CCRC) and pheochromocytomas (PHEO).

It's well known that patients that present microdeletions/insertions, nonsense and deletion mutations usually do not develop PHEO (type 1) but do develop RA, HB and CCRC.

In patients with missense mutations, PHEO can be present (type 2) even as the only feature, or not present. One way to improve our knowledge about the correlation between missense mutations and the phenotype associated is the finding of more and new mutations in families.

We have studied 17 different families with missense mutations (12 different mutations) and their correlation with phenotype. Eight of these mutations fit well with the phenotype previously described: PHEO was present in patients with G114S, R161Q, R167Q (5 families), R167W, Y175C mutations, and PHEO was not present in patients with N78S (2 families), L128R and L178P mutations. Three mutations showed discrepancies with the phenotype previously described according to the presence or absence of PHEO (T157I, Q164R and L184P). We also describe the mutation X214R not reported previously, which was present in a patient with bilateral PHEO. This mutation probably extends protein by an additional 14 aminoacids.

These data support previous associations in eight mutations, add details about genotype-phenotype correlation in three mutations and give information about the phenotype associated to X214R mutation.

Databases searched:

The UMD-VHL Locus Specific Database: <http://www.umd.be:2020/>  
The HGMD professional release 7.4: <http://www.hgmd.cf.ac.uk/ac/index.php>

## P05. Molecular and biochemical basis of disease

**P05.001****ABCB4 gene mutations and differential involvement in liver diseases**

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ABCB4 gene mutations are responsible for type 3 Progressive Familial Intrahepatic Cholestasis in children (PFIC3), while a possible role in idiopathic cholangiopathies in adults has not been explored. We know that absence or deficiency of the floppase activity necessary for biliary excretion of phosphatidylcholine, leading to lack of phospholipid protection in the bile against the detergent action of bile acids and damage of the biliary epithelium. Our aim was to unveil the role of ABCB4 gene in clinically relevant hepatobiliary diseases in children and adults.

We collected DNA samples from 168 patients (80 children with PFIC3 phenotype, 41 women with Intrahepatic Cholestasis of Pregnancy (ICP), 16 adults with idiopathic cholestasis, 27 adults with primary sclerosing cholangitis (PSC), 4 patients with juvenile cholelithiasis), 150 healthy subjects including 50 two-parous women without ICP. Molecular analysis of ABCB4 gene has been so far completed in 110 patients and 100 healthy subjects.

We observed 52 ABCB4 mutations in 32 patients: 37 were found in 19 PFIC3 children, 5 in 5 ICP women, 7 in 5 patients with PSC, 2 in 2 adults with idiopathic cholestasis and 1 in one patient with juvenile

cholelithiasis. Eleven mutations (9 in PFIC3) cause truncated protein while 41 mutations (12 in adult) cause single amino acid change.

The relative low percentage of patients which co-segregates with ABCB4 mutations suggests a genetic heterogeneity for PFIC3 disease and a possible role of ABCB4 as a modifier or gene susceptibility in adults with chronic or transient cholangiopathies triggered by a cholestatic injury.

**P05.002****The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression**

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Mutations in Autoimmune Regulator protein (AIRE) cause autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). AIRE is expressed in thymic medullary epithelial cells where it promotes the expression of tissue-restricted antigens. By the combined use of biochemical and biophysical methods we show that AIRE selectively interacts with histone H3 through its first PHD finger (AIRE-PHD1). AIRE-PHD1 discriminates between different degrees of histone H3 lysine 4 (H3K4) methylation and preferentially binds non-methylated H3K4 (KD ~4 uM). Accordingly, *in vivo* AIRE binds and activates promoters containing non-methylated H3K4 in HEK293 cells. We propose that AIRE-PHD1 is a prototype of a new class of PHD fingers that specifically recognize non-methylated H3K4, thus providing a new link between the status of histone modifications and the regulation of tissue-restricted antigen expression in thymus.

**P05.003****The co-localization of ASC with A $\beta$  fibrils in post-mortem brain samples of Alzheimer's disease patients**

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Amyloid is an extracellular insoluble protein aggregate which accumulates in several tissues in various clinical conditions. The co-localization of ASC (Apoptosis associated Speck like protein containing a Caspase recruitment domain), a key molecule in both apoptotic and inflammatory processes, with AA type amyloid fibrils has previously been demonstrated by our group. ASC is known to form cytosolic aggregates called specks. The aim of this study was to determine whether the distribution of ASC is altered around A $\beta$  deposits and senile plaques in post-mortem brain samples of Alzheimer's disease patients. Immunohistochemical staining of paraffin-embedded tissues from post-mortem brain samples of 12 Alzheimer's disease patients revealed co-localization of ASC protein with senile plaques. This co-localization was confirmed by ASC-A $\beta$  co-staining by using immunofluorescence staining technique. To investigate whether ASC expression was correlated with amyloid deposition, sequential sections from AD patients were analyzed after congo red staining. There was a strong correlation between ASC expression and the presence of amyloid deposits. We further investigate the interaction between ASC and A $\beta$  in ASC-YFP and APP (Amyloid Precursor Protein) co-transfected COS-7 cells which also demonstrated that specks are located near the intracellular A $\beta$  deposits. We hypothesize that expression of ASC may be important in the pathogenesis of A $\beta$  amyloid formation and in senile plaque development in predisposed tissues.) Further functional studies are required to explore the link between ASC and A $\beta$  amyloid formation.

**P05.004****A new candidate haplotype in the ps2 gene associated with late-onset familial alzheimer disease?**

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Mutations within the APP, presenilin 1 (PS1) or presenilin 2 (PS2) genes are found in familial Alzheimer disease (AD). Whereas mutations within PS1 cause AD of early or very early onset, APP as well as

PS2 mutations are responsible for both early and late onset familial AD. Promoter and intronic polymorphisms have been detected on both PS genes and some of those have been reported as risk factors for sporadic AD development.

With the aid to detect PS2 mutations in four unrelated late-onset AD patients with familial history we sequenced PS2 exons 3 - 12. Primers were designed to amplify each exon and its surrounding sequences. Since intron 9 is relatively small exons 9 and 10 were coamplified. No mutation within the coding region was detected, instead all four patients carried the haplotype TGTGG corresponding to polymorphisms C129T on exon 3, A(-24)G on intron 3, C261T on exon 4, G(+160)C polymorphism on intron 9 and A(+24)G on intron 11. The polymorphism G(+160)C, located on the shortest of all PS2 introns, had not been identified before. The additional genotyping of fifty non-demented age-matched control individuals revealed that the TGTGG haplotype is present at a very low frequency (2%) in the normal population. To conclude, the haplotype constituted by five different polymorphisms on the PS2 gene described in the present study seems to be associated with familial AD. The analysis of further familial late-onset AD cases will reveal its importance as diagnostic marker.

#### P05.005

#### Mutation detection in ENAM and MMP20 genes in amelogenesis imperfecta

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Amelogenesis imperfecta (AI) is one of the common of inherited tooth disorders. AI is distinguished by abnormality in enamel formation. The frequency of this disease is different through the worldwide. Studies have demonstrated that several genes were associated with AI such as ENAM and MMP20 genes. MMP20 gene is part of a cluster of matrix metalloproteinase (MMP) genes. Protein of the (MMP) family is involved in the breakdown of extracellular matrix in normal physiological processes. ENAM is another gene and it plays crucial role in normal teeth development. The aim of this investigation was to study mutation detection in 10 Iranian families with non-syndromic AI.. We carried out a polymerase chain reaction (PCR) and single-stranded conformation polymorphism (SSCP) for mutation detection within ENAM and MMP20 genes. SSCP analysis of genomic DNA from the AI family revealed the presence of an abnormal conformer in the AI patients. DNA Sequencing revealed the presence of mutation in the different part of the subjected. We found genetic changes in patients with different type of inheritances, one patient in exon 4 of MMP20 gene and one patient for ENAM exon 9. The data presented here is in agreement with the previous studies that suggested these genes are associated with tooth disorder. Taken together these findings support MMP20 and ENAM as disease genes, and opened a new window on the molecular mechanism of the AI disease and to the function of the enamelin protein in enamel formation.

#### P05.006

#### The role of ATM in monitoring the integrity of mitotic spindle

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Centrosomes are cytoplasmic organelles that organize the interphase cytoskeleton and contribute to bipolar spindle formation during mitotic cell division. Dysfunction of the centrosome/centriole regulatory controls can generate supernumerary centrosomes, abnormal mitotic spindles and finally chromosomal instability.

We previously showed that during the cell cycle progression ATM is activated by phosphorylation at Ser1981 at each mitosis and localizes at centrosomes together with p53 phosphorylated at Ser15, so as to keep it inactive at centrosomes when the spindle is correctly in place. In case of disruption of mitotic spindle, as the result of nocodazole damage, the colocalization of ATM and p53 is lost at centrosomes but present in form of spots dispersed in the cytoplasm (Oricchio et al., 2006).

Here we show that in ATM-defective cells (both lymphoblasts and peripheral blood lymphocytes from AT patients) p53 does not colocalize

at centrosomes with gamma-tubulin. In contrast, the colocalization of p53 and gamma-tubulin takes place in Mre11-defective cells established from an AT-LD patient. In conclusion, the monitoring of the mitotic spindle appears to be an ATM-dependent phenomenon.

Oricchio E., Saladino C., Iacovelli S., Soddu S., Cundari E. Cell Cycle 5/1, 88-92, 2006

#### P05.007

#### Incidence of NLGN genes in greek autistic patients

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Autism and Autism Spectrum Disorders (A.S.D.) belong to the group of neurodevelopmental disorders with a prevalence of 5-10/10,000 and male to female ratio 3-4:1.

This study aimed to analyse the Neuroligin 3 gene (*NLGN3*, Xq13) and Neuroligin 4 gene (*NLGN4*, Xp22.3) in patients with A.S.D. The sample includes 367 individuals of Greek origin (169 patients, 154 mothers, 44 first-degree relatives). All patients had been diagnosed with A.S.D. by neurologists, psychiatrists and clinical geneticists according to the DSM-IV criteria. Patients carrying chromosomal aberrations or Fragile X syndrome were excluded from the study. The mutation p.Y74Y of *NLGN3* was examined with ARMS PCR whereas mutations p.R451C of *NLGN3* and p.1186insT of *NLGN4* were screened by dHPLC. Mutations p.Y74Y and p.R451C were not identified in our samples. However, the dHPLC screening for the p.1186insT mutation suggested the existence of a mutation in two samples (in a patient and his mother). Sequencing revealed the c.1597A>G mutation (p.K378R) in *NLGN4*. This gene is thought to play an important role in synaptogenesis and synapse remodelling in the neuronal circuitry of the brain, representing a good functional candidate for A.S.D. This is the first molecular study of individuals with A.S.D. in Greece. Sequencing of the rest of the exons of our samples may allow for a genotype-phenotype correlation in the Greek population.

#### P05.008

#### Auto-brewery syndrome - genetic testing

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The concentration of ethanol in blood, expired air and urine constitutes important proof to accuse drunk drivers. However the reliability of alcohol concentration analysis results is very often questioned by the lawyers. One of the argument is the opinion that alcohol could be produced in the body, due to "auto-brewery" syndrome - increased level of blood alcohol caused by yeast or bacterial fermentation in the small intestine. Moreover, endogenous alcohol is produced in blood even after human death. Our aim was to prepare genetic test to confirm or exclude presence of microbial flora of intestine and blood. DNA was isolated from blood and intestinal content samples collected during autopsies of car accidents victims and was used in PCR reaction with universal primers targeting the conserved regions ITS1 and ITS2. Autopsy samples were compared with known fungal strains. The results induced us to propose one hybridization fluorescent probe combined with Cy5 and LNA nucleotides. The probe works with LightCycler system in one reaction with SYBR Green and allows to detect specifically 6 common yeast species. The method seems to be fast, cost-effective and decisive in cases of judicial doubts.

#### P05.009

#### Role of PHD fingers and COOH-terminal 30 amino acids in AIRE transactivation activity

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Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autosomic autoimmune disease resulting from the defective function of a gene codifying for a transcription factor named autoimmune regulation (AIRE). The AIRE protein contains several domains among which two PHD fingers involved in the transcriptional

activation. We investigated the function of the two PHD finger domains and the COOH terminal portion of AIRE by using several mutated constructs transfected in mammalian cells and a luciferase reporter assay. The results predict that the second PHD as well as the COOH terminal regions have marked transactivational properties. Our studies indicate a prevalent role of the second PHD since the C446Y mutation, which alters the PHD2 sequence led to a complete loss of the transactivation activity.

The COOH terminal region contains the fourth LXXLL and the PXX-PXP motifs which play a critical role in mediating the transactivation capacity of the AIRE protein. On the other hand, the crucial role of the PXXPXP sequence has already been defined by the finding of the disease-causing mutation in the sequence of APECED patients. Our study provides a definition of the role of the PHD fingers in transactivation and identifies a new transactivation domain of the AIRE protein localized in the COOH terminal region.

#### P05.010

#### Homozygosity mapping with SNP arrays as a useful technology for diagnosis in complex diseases as Bardet-Biedl syndrome in consanguineous families

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Bardet-Biedl syndrome (BBS, MIM 209900) is a rare pleiotropic human genetic disorder that has as primary phenotypic features: early-onset retinitis pigmentosa, obesity, renal abnormalities, limb abnormalities and also a variable degree of cognitive impairment.

This disorder is genetically heterogeneous with twelve genes identified (BBS1-BBS12). BBS also shows considerable inter- and intra-familial variation of the phenotype. To date mutation screening has resulted in the identification of approximately 70% of the causative mutations, indicating that additional BBS genes have to be identified.

At the moment is very hard and time consuming to search for mutations in each of the genes involved, as some of them have been implicated in a very low percentage. In this study we employed high-density SNP genotyping for homozygosity mapping in the identification of gene mutations to simplify this task in consanguineous families.

Nine consanguineous families were analyzed, and in five of them linkage to a known BBS loci was detected. Sequencing of the BBS gene that localizes in the locus where linkage was detected, revealed 5 new mutations (G2X, BBS3; L454P and G250R, BBS6; P108 and del 2pb in 372, BBS12). Cosegregation of the mutation in the family corroborates the pattern of autosomal recessive inheritance.

For the rest of the families linkage indicated several novel candidate BBS gene loci.

#### P05.011

#### Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome

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Meckel-Gruber syndrome (MKS) is a genetically heterogeneous, neonatal lethal malformation and the most common form of syndromic neural tube defects (NTDs). To date, several MKS genes have been identified, whose protein products affect ciliary function<sup>1-5</sup>. Here we show that mutations in MKS1, MKS3 and NPHP both cause Bardet-Biedl syndrome (BBS) and also have a potential epistatic effect on mutations in known BBS loci. Five of six families with MKS1 and BBS mutations manifested seizures, a feature that is not a typical component of either syndrome. Functional studies in zebrafish showed that mks1 is necessary for gastrulation movements and that it interacts genetically with known bbs genes. These observations are not restricted to

MKS1. We also found two families with missense or splice mutations in MKS3, one of which also bears a homozygous nonsense mutation in NPHP6 that likely truncates the extreme C-terminus of the protein. These data extend the genetic stratification of ciliopathies and suggest that BBS and MKS, although clinically distinct, are allelic forms of the same molecular disorder.

#### P05.012

#### Bartter syndrome: think of CLCNKB

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Bartter syndrome (BS) is an autosomal recessive disorder characterized by renal salt wasting and hypokalaemic metabolic alkalosis. The primary defect is a reduced NaCl reabsorption in the thick ascending limbs of Henle's loop. Four types of recessive BS exist: congenital without (I/II) or with sensorineuronal deafness (IV) and the milder form (III). Mutations in SLC12A1, KCNJ1, CLCNKB and BSND give rise to BSI-IV, respectively. Here, we have analyzed CLCNKB in a group of KCNJ1-mutation negative BS patients (n=60).

More than half of the published mutations in BSIII are (partial) CLCNKB deletions, caused by unequal cross between the homologous CLCNKA and CLCNKB. Therefore, we have performed multiplex ligation-dependent probe amplification analysis of CLCNKB. Deletion of the entire gene has been observed homozygotously in four and heterozygotously in 2 patients. Three patients have a heterozygous deletion of the 5' end of the gene (promoter region through exon 8). Additionally, sequence analysis revealed a second mutation in the five patients with a heterozygous deletion. In the remaining patients, we have found seven patients with two mutations (both in the homozygous or compound heterozygous state), but also 7 patients with only one mutation. It is unclear whether the second mutation is present elsewhere in the CLCNKB gene, or whether one of the other genes involved in BS is mutated.

Together, BS type III has been confirmed in 16 of the 60 BS patients. Since we have found mutations in KCNJ1 in 10 patients, we suggest that BSIII is more prevalent than BSII.

#### P05.013

#### Exploring the contribution of Conserved Non-Coding sequences (CNCs) to Blepharophimosis Syndrome (BPES)

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Blepharophimosis syndrome (BPES) is a development disorder caused by FOXL2 mutations, total gene deletions or extragenic deletions. In 12% of patients however, the molecular defect remains unknown. We hypothesise that copy-number variations (CNVs) or point mutations in cis-regulatory regions mapping within the minimal extragenic deletion region could affect FOXL2 transcription and cause disease.

In a panel of 33 molecularly unresolved BPES patients, one novel extragenic deletion upstream of FOXL2 was found by array CGH. This deletion overlaps with the previously defined shortest region of overlap (SRO) of upstream extragenic deletions, and was confirmed by qPCR. In addition, qPCR of 25 CNCs located in this SRO revealed putative CNVs in 9 patients, of which the significance is being evaluated. Moreover, sequencing of the 25 CNCs revealed 4 putative pathogenic variants, which are further being investigated by luciferase assays.

Second, we characterized 9 known deletions of the *FOXL2* region using array CGH. In addition, 10 new deletions identified by MLPA were further defined by qPCR. These deletions prove to be highly heterogeneous with regard to deletion size and breakpoint localization. They account for 12% of molecular defects in BPES, highlighting the importance of copy number analysis in BPES.

In conclusion, we showed that copy number changes of *FOXL2* comprise a considerable fraction of the molecular defects in BPES. Moreover, the extensive search for CNVs and point mutations in CNCs mapping distantly of *FOXL2* in BPES patients, support the importance of long distance gene regulation in the molecular pathogenesis of this disorder.

#### P05.014

##### Molecular analysis of the *ATP6V1B1* and *ATP6V0A4* genes in distal renal tubular acidosis

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Primary distal renal tubular acidosis (dRTA) is characterized by defective secretion of H<sup>+</sup> ions by intercalary cells of the collecting duct. Both autosomal dominant (AD) and recessive (AR) forms have been described. The AD form is caused by mutations of the *SLC4A1* gene. The AR form has been associated with mutations of either *ATP6V1B1* - in individuals who usually display sensorineural hearing loss (SNHL) - or *ATP6V0A4* - in individuals who do not have SNHL or show hearing loss only after the first decades of life.

We report on the first investigation of the *ATP6V1B1* and *ATP6V0A4* genes in dRTA patients in Italy. Mutations were identified in 8/8 patients analyzed.

The *ATP6V0A4* Arg807Gln mutation was found at the homozygous state in a patient with severe early-onset SNHL, that is generally not observed in subjects with *ATP6V0A4* defects. Only another case with the same genotype, who had a comparably severe phenotype, is reported in the literature.

A monoallelic *de novo* *ATP6V1B1* mutation (Arg394Gln) was observed in one patient with a typical dRTA renal phenotype without deafness. This mutation has been previously described in two cases, who also were apparently simple heterozygotes.

We also detected three previously unreported mutations, two in *ATP6V0A4* and one in *ATP6V1B1*.

Our results shed further light on phenotype-genotype correlations in dRTA. In particular, the association of a specific monoallelic *ATP6V1B1* mutation with a dRTA phenotype without hearing loss suggests that other genetic mechanisms, in addition to autosomal recessive inheritance, may be associated with *ATP6V1B1* alterations.

#### P05.015

##### An *EYA1* gene mutation in intron 8 [c.867+5G>A] causes alternative RNA splicing and is a recurrent mutation causing Branchial-oto-renal syndrome

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Branchio-oto-renal (BOR) syndrome is a heterogeneous autosomal dominant disorder characterized by branchial arch abnormalities, hearing loss and renal abnormalities. Variable expression of clinical features is found within and between affected families. Mutations in the *EYA1* gene are reported to account for 40-70% of reported BOR syndrome cases. We have developed a strategy for molecular testing of the *EYA1* gene causing BOR syndrome consisting of 1) sequencing of the complete coding region and flanking intronic regions and 2) multiple ligation probe amplification (MLPA) analysis. Using this strategy *EYA1* mutations were identified in 82% (14/17) of a cohort of paediatric BOR syndrome probands. Forty-five percent (5/11) of BOR syndrome probands in our cohort had *de novo* *EYA1* mutations, suggesting that the incidence of *de novo* cases in BOR syndrome is higher than 10%

previously reported. We also describe a previously unreported recurrent *EYA1* gene mutation c.867+5G>A found in 21% (3/14) of *EYA1* mutation-positive BOR probands in our patient cohort. RNA analysis indicates that the c.867+5G>A mutation affects splicing of the *EYA1* gene, and produces an aberrant mRNA transcript skipping exon 8 and leading to a premature termination signal in exon 9. The aberrant transcript lacking exon 8 was present at approximately 50% level of wild-type *EYA1* mRNA in fibroblasts, and suggests that certain transcripts of *EYA1* escape nonsense-mediated decay and encode truncated EYA protein capable of dominant-negative interactions causing disease.

#### P05.016

##### BSCL2 and its possible function in adipogenesis

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Congenital generalized lipodystrophy, first described by Berardinelli (1954) and Seip (1959), is a rare autosomal recessive disorder characterized by near complete absence of adipose tissue from birth or early infancy. Two distinct forms can be distinguished: CGL1 and CGL2, whereas CGL1 is caused by mutations in AGPAT2 and CGL2 by mutations in BSCL2. Whilst mutations in AGPAT2, a member of the acyltransferase family, likely cause lipodystrophy by reducing triglyceride synthesis in adipose tissue, the pathogenic effects of mutations in BSCL2 on a molecular level are not yet fully understood. Further clinical features of both subtypes include severe insulin resistance, acanthosis nigricans, muscular hypertrophy, hepatomegaly, diabetes mellitus and hypertriglyceridemia. However patients with CGL2 show a more severe phenotype with lack of both metabolically active and mechanical adipose tissue, a higher prevalence of mild mental retardation and hypertrophic cardiomyopathy.

To elucidate the function of BSCL2 in adipogenesis we generated BSCL2 knock-out flies. These knock-out strains showed altered architecture and distribution of lipid droplets in adipose tissue. Therefore our data suggest that BSCL2 is involved in lipid droplet formation and/or maintenance in Drosophila. To confirm these data in mammals we created a mouse knock-out model for BSCL2. We chose the Cre-loxP system to obtain conditional knock-out mice in order to investigate the function of BSCL2 in relevant tissues specially during adipocyte development. We will apply molecular genetic, histological, biochemical and cell culture methods to further determine the enigmatic role of BSCL2 in adipogenesis and in the pathophysiology of CGL2.

#### P05.017

##### The functional significance of the C1 inhibitor gene promoter mutations

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C1 inhibitor (C1INH) is a key negative regulator of complement activation. Decreased level of functional C1INH causes hereditary angioedema which is usually transmitted as an autosomal dominant trait. Till now, three promoter mutations have been detected in the C1 inhibitor gene (C1NH), one of them (-45 C>A) having been newly identified in our laboratory. Mutations -40 C>G and -45 C>A both appeared to cosegregate with other mutations in coding region, while the third mutation -103 C>T did not. Interestingly, in patients suffering from hereditary angioedema, mutation -103 C>T was found exclusively in homozygous state while heterozygous individuals were healthy. The objective of our work was to assess functional importance of the three promoter mutations on the C1NH gene expression. Results of luciferase reporter gene assay showed that two of the mutations, -40 C>G and -45 C>A, have negligible effect on C1INH expression. Surprisingly, mutation -103 C>T increased luciferase gene expression by 59 % in comparison to wild type promoter construct. Yet plasma levels of C1 inhibitor in patients with this mutation were significantly decreased. The reason for this discrepancy is not obvious. However, a negative correlation between amount of mRNA and plasma levels of C1INH has already been described in healthy controls. Such a correlation suggests that a negative feedback regulation of C1INH expression may exist.

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**P05.018****Screening for CADASIL in central Italian patients**

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Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is an inherited autosomal dominant condition characterized by a variable combination of recurrent cerebral ischemic episodes, cognitive deficits, migraine with aura and psychiatric symptoms. It results from mutations distributed throughout the 34 EGFRs (epidermal growth factor-like repeats) of the Notch3 gene, leading to the addition or the loss of a cysteine residue.

We performed genetic testing for Notch3 mutations in 45 probands of different family with suspected CADASIL who had been referred to our service in 2007. DNA samples were analyzed for mutations in all exons of EGFRs using an appropriate screening protocol considering the mutations distribution in exons gene.

The sequence analysis of Notch3 gene revealed the presence of three previously reported missense mutations: C144F in exon 4, G528C in exon 10 and R1006C in exon 19. In particular, the mutation C144F was found in one family, whereas the mutations G528C and R1006C were found in two and seven families respectively, with the same regional origin. These results suggest a higher frequency of exon 10 and 19 mutations in central Italy and confirm that the genetic procedures could be optimized by a geographical region-oriented genetic analysis.

We are extending genetic analysis to the remaining exons of Notch3 gene in patients with clinical and strumental features more suggestive of CADASIL phenotype, negative for EGFRs mutations.

**P05.019****KRIT1 gene mutations in Sardinian patients with cerebral cavernous malformations**

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Cerebral Cavernous Malformations (CCM) are CNS vascular anomalies associated with seizures, headaches and hemorrhagic strokes and represents 10 to 20 percent of cerebral lesions. CCM is present in 0.1 to 0.5 of the population. This disorder most often occurs sporadically but may also be familial. Familial cases are inherited as a dominant trait with incomplete penetrance and are estimated to account for 10-40 % of the patients. The identification of the genes involved in such disorders allow to characterize carriers of the mutations without clear symptoms. The first gene involved in CCM1 is KRIT 1. In addition to KRIT1 two other genes have been described: MGC4607 (CCM2) and PDCD10 (CCM3). We selected 14 patients belonging to seven Sardinian families on the basis of clinical symptoms and Magnetic Resonance results. MLPA analysis of KRIT1, MGC4607 and PDCD10 gave negative results. Sequencing analysis of KRIT1 gene was performed in all the patients. We identified a 4bp deletion in exon 9 leading to a premature stop codon in a patient with clear phenotype. The same mutation has been found in three relatives showing very mild symptoms. In 5 subjects belonging to four unrelated families a unique nonsense mutation (C329X) has been found. Haplotype analysis in these four families revealed a common origin of the mutation. These data suggest a "founder effect", already described in different populations.

**P05.020****Functional analysis of CD96, a causative gene for a form of C (Opitz trigonocephaly) syndrome**

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gasaki, Japan, <sup>10</sup>Health Sciences University of Hokkaido, Tobetsu-cho, Japan.

The C syndrome is characterized by trigonocephaly associated with unusual facies, psychomotor retardation, redundant skin, joint and limb abnormalities, and visceral anomalies. In an individual with the C syndrome harboring a balanced chromosomal translocation, t(3;18)(q13.13;q12.1), we identified a gene (CD96), which encodes a member of the immunoglobulin superfamily, was disrupted at the 3q13.3 breakpoint. In mutation analysis of karyotypically normal patients with the C or C-like syndromes, we identified a missense mutation in exon 6 of the CD96 gene in one patient and found reduced expression in two patients. In order to know the function of CD96, we established normal CD96 or the mutated CD96 expressed cell lines and investigated their characters especially on cell-adhesion activity and cell-growth activity. Cells with the normal CD96 protein increased both the cell-adhesion and growth activities compared with MOCK cells. On the other hand, cells with the mutated CD96 protein lost the activities in vitro. These findings may indicate that CD96 mutations cause a form of the C syndrome by interfering with cell adhesion and growth.

**P05.021****Evidence on microRNA-mediated regulation of CDK5R1 gene expression**

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CDK5R1 encodes for p35, an activator of CDK5, which is involved in neuronal migration and differentiation during central nervous system development and has been candidated for mental retardation. We recently reported that the large 3' untranslated region (3'UTR) of CDK5R1 contains regulatory elements affecting transcript stability. Besides several AREs, 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, including two neuroblastoma derived lines. Among the expressed miRNAs, we observed that miR-15a, miR-103 and miR-107 presented a high number of target sites with a free energy <-20 kcal/mol. A preliminary quantitative analysis of the three above miRNAs and p35 showed an inverse correlation between miR-107 and p35 expression, suggesting a negative effect of miR-107 on CDK5R1 expression. Overexpression of miR-107 by transfection of the specific precursor in neuroblastoma SK-N-BE cells showed, after 72 hours, a 45 and 75% decrease in p35 expression respectively using 50 and 100 nM of the precursor.

Transfection of anti-miR-107 will be tested. Luciferase constructs will be used to validate the predicted miRNA target sites in CDK5R1 3'UTR. These preliminary data allow us to hypothesize a role of miRNAs in post-transcriptional CDK5R1 regulation.

**P05.022****Expression of ceruloplasmin and ferroportin in human peripheral blood lymphocytes: a new link between iron metabolism and the immune system**

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**Background:** Ceruloplasmin (Cp) is a multicopper oxidase with a relevant role in iron (Fe) metabolism mainly due to its ferroxidase activity. Cp exists in a secreted form (sCp) and a membrane glycosylphosphatidylinositol-anchored form (GPI-Cp). Recent studies showed that Cp ferroxidase activity is required for ferroportin (Fpn) stability on cell surface. Herein we report the expression of both Cp isoforms and Fpn in human peripheral blood lymphocytes (PBL).

**Material and Methods:** PBL were isolated followed by total RNA extraction, cDNA synthesis and subsequent analysis of Cp and Fpn transcripts by PCR. For immunoblotting, protein extracts from membrane and cytosolic fractions were prepared. For immunofluorescence, cells were cultured with and without PI-PLC (an enzyme that cleaves GPI groups) followed by immunolabeling for Cp and analysis by confocal microscopy.

**Results and Conclusion:** Both Cp isoforms and Fpn were shown to be expressed at both mRNA and protein level. Cp and Fpn were also shown to be localized at PBL membrane. Confocal analysis of immunolabeled PBL treated with PI-PLC showed a significant reduction of Cp labeling compared to untreated PBL, showing that at least part of

Cp expressed at PBL membrane is GPI-anchored. Altogether, these data show that human PBL express both sCp, GPI-Cp and Fpn. Studying the expression of these proteins in cells of the immune system (IS) may contribute to understanding the regulation of the Fe homeostasis by the IS and also allow to access Cp isoforms and Fpn activities associated to pathological conditions, often neurological, in cells from peripheral blood.

#### P05.023

##### **Myotonia congenita: identification of eleven new mutations in the CLCN1 gene**

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**Introduction.** Myotonia congenita (MC) is a rare autosomal inherited disorder characterized by muscle stiffness and hypertrophy. MC is inherited in two different forms: a dominant form, designated as Thomsen's myotonia, or a recessive form, designated as Becker's generalized myotonia. However, both forms are caused by mutations in a unique gene, the CLCN1 gene, which encodes the major chloride channel in skeletal muscle. In this communication, we identified 11 new mutations in Spanish patients. We assumed these are disease-causing mutations and no polymorphisms because the mutated amino-acid residues are conserved through evolution. Our future project is to characterize, using patch-clamp analysis, the effect of these mutations on the clc1 channel function.

**Patients and Methods.** We analyzed DNA extracted from peripheral blood of patients attending to Neurology Services from several tertiary Spanish hospitals. Each CLCN1 exon and boundary intron regions were amplified by the DNA polymerase chain reaction (PCR) and products were DNA sequenced in an ABI Prism® 3130 XL instrument.

**Results.** We analyzed DNA from 103 patients (51 families) with myotonia as major symptom having previously excluded the myotonic dystrophies. We have found 20 different mutations, including 11 mutations that are not described before. Four mutations (F167L, Y302H, M485V and R894X) were recurrent in our serie.

**Conclusions.** Analysis of mutations in CLCN1 gene in 51 Spanish families with myotonia suggest that there are remarkable differences in the mutation profiles between Spanish and other Caucasian populations.

#### P05.024

##### **A silent mutation within protectin (CD59) gene and exon skipping in a family with coeliac disease**

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Protectin (CD59), which inhibits the assembly of the membrane attack complex following complement activation, was reported to be upregulated in coeliac disease [1]. Its gene is located on chromosome 11p12, to which suggestive linkage was observed in a Maltese family with coeliac disease.

Sequencing of CD59, APAF-1 interacting protein and CD44 genes, revealed a number of variants. Two synonymous variants, a C/T transition (rs1071695) in CD44 and a novel G/A change within CD59, were co-segregating with the linked haplotype in all affected individuals. Only one A allele, from 442 chromosomes, was found in the general population (0.23%) and was completely absent in a group of coeliac patients, showing that this is a rare variant found within this family. Two constructs consisting of normal and mutated exons together with adjacent introns were cloned into pSPL3, and transfected into HeLa and COS-7. Reverse transcriptase-PCR was performed, followed by agarose gel electrophoresis and sequencing. Both wild-type and mutated constructs for the CD59 variant resulted in a normally and an abnormally spliced transcript lacking the exon involved (coding for signal peptide), which was more pronounced in the presence of the A allele. No abnormally spliced transcripts were observed for the other variant within CD44.

In this study, a novel synonymous variant within the CD59 gene was shown to affect splicing, possibly due to effects on the secondary structure of pre-mRNA, increasing the susceptibility to coeliac disease in this family.

1. Berstad, AE & Brandtzaeg, P. Gut. 1998; 42: 522 - 529

#### P05.025

##### **In depth study of hCYP21A2 in Spanish General Population: High frequency of gene duplications and sequence variations**

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21-hydroxylase deficiency (21OHD) is one of the most common autosomic recessive disorders. The 21-hydroxylase enzyme is encoded by *hCYP21A2* gene, which is highly homologous to the *hCYP21A1P* pseudogene. The high variability of the *CYP21A2* locus hinders the characterization of 21OHD alleles and complicates disease carrier detection and genetic counselling. We present the study of *hCYP21A2* in 288 Spanish population chromosomes to estimate frequency of 21OHD carriers, *hCYP21A2* duplications and *hCYP21A2* novel variations.

*hCYP21A2* was sequenced after PCR amplification in two fragments and *hCYP21A2* dosage was done by Real-Time PCR. Haplotype construction was based on a Bayesian method using 20 polymorphisms all over *hCYP21A2*.

**Copy number variations.** 3.5% of alleles carried two copies of *hCYP21A2* associated mostly with p.Gln318X.

**Frequency of putative disease-causing alleles.** 23 *hCYP21A2* mutations were found (8% of alleles): 17 are known to allow 30-60 % of the WT activity, 2 completely impairs enzyme activity, and 4 were novel, which effect on the activity is unknown.

***hCYP21A2* polymorphism.** 79 different variations were found distributed all over the gene and its close vicinity.

**Haplotypes.** 75 different haplotypes were identified, most appearing only once, but some recurrent.

This study shows the highest frequency of 21OHD carriers reported by genotyping study. Furthermore, it showed a high frequency of *hCYP21A2* duplications with one of the copies mutated and also high frequency of novel mutations with unknown effect on the 21OH activity. These novel variations as well as gene duplications should be considered when doing the genetic 21OHD diagnosis and genetic counselling.

#### P05.026

##### **Inactivating mutations in the ABCC8 gene in patients with congenital hyperinsulinism of infancy (CHI)**

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Congenital Hyperinsulinism of Infancy (CHI) is a genetically heterogeneous disease characterized by severe hypoglycemia due to excessive insulin secretion from pancreatic β-cells. Two histopathologically and genetically distinct groups are recognized among patients with CHI. A diffuse form, which involves the entire pancreas, arises predominantly from autosomal recessive inheritance. A focal form, which shows localized adenomatosis of islet cells within the normal pancreas, arises from a germline mutation of the paternal allele in addition to somatic loss of the maternal allele in adenomatous pancreatic β-cells. The most common cause of CHI are inactivating mutations in the genes coding for the two subunits of the β-cell ATP-sensitive K<sup>+</sup> channel, ABCC8/SUR1 (regulatory subunit) and KCNJ11/Kir6.2 (pore-forming subunit). We examined 64 children with a diffuse, focal, atypical or unknown form of CHI and sequenced the entire coding region and the exon/intron boundaries of the ABCC8 and KCNJ11 genes. We found 34 mutations (4 homozygous, 7 compound heterozygous, 23 heterozygous) in the ABCC8 gene (53%) and 2 heterozygous mutations in the KCNJ11 gene (3%). In 28 patients (43%) no mutations were identified (see table). Three patients with a heterozygous mutation in the ABCC8 gene and diffuse CHI inherited the mutation from the mother. We missed the second paternal mutation with our screening method. Most likely, the low detection rate is caused by missed mutations in ABCC8/KCNJ11 and further locus heterogeneity.

## ABCC8 mutations in 64 children with CHI

Form of CHI	diffuse	focal	atypical	unknown	total
homozygous	3	0	0	1	4
compound heterozygous	7	0	0	0	7
heterozygous	10	9	0	4	23
no mutation	18	1	1	8	28
total	38	10	1	13	62

## P05.027

## Homozygosity mapping of congenital hyperinsulinism of infancy (CHI) in Italian patients

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Congenital hyperinsulinism of infancy (CHI) is a heterogeneous disorder due to genetic mutations 80% of which are found in genes encoding both subunits of pancreatic KATP channel ABCC8 and KCNJ11. Other causative genes have been identified but, up-to-date, in 30% of patients the genetic basis of CHI has yet to be determined, suggesting additional locus heterogeneity.

SNP microarrays have improved the possibility for autozygosity mapping of rare disorders with founder mutations, in order to identify disease susceptibility loci.

In our study we have performed a genome wide SNP mapping in twenty two CHI probands and their families using Affymetrix GeneChip® 250K Human Mapping arrays. Using a complex computational approach, we identified long contiguous stretches of copy number neutral-homozygous loci (autozygosity) on chromosomes 1, 5, 7, and 8, both in consanguineous and in non consanguineous families. Three patients, two of which carried a causative mutation on ABCC8/KCNJ11 genes, showed a wide common region of homozygosity on chromosome 11p15. Additionally, two probands shared stretches of homozygosity on chromosomes 4, 6, 10 ,11 and 18.

Moreover, one patient, negative for ABCC8/KCNJ11 gene mutations, showed a homozygous region of 79Mb on chromosome 4 containing the causative gene HADH. Direct sequencing of the coding region of HADH gene showed a homozygous C to T transition in exon 6 leading to a premature stop of the synthesized protein. Further refinement of other candidate regions by microsatellites marker analysis and linkage analysis will be necessary to map other disease loci in CHI families.

## P05.028

## Congenital Nephrotic Syndrome (CNS) caused by a complex genotype in the Nephrin gene (NPHS1) revealing 2 novel mutations

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Nephrotic syndrome (NS) is characterized by gross proteinuria, hypoalbumenia, edema and hyperlipidemia, which in CNS manifest in utero or within 3 months after birth. Inherited impairments of the glomerular filtration barrier are an important cause of NS, and in CNS and infantile NS ~60% of cases are caused by mutations in 4 genes: Nephrin (NPHS1), Podocin (NPHS2), Laminin-β2 (LAMB2) and Wilms' Tumor suppressor gene (WT1). In a newborn with clinical findings compatible with CNS, and his parents, the 27 exons of the NPHS1 gene and 8 exons of the NPHS2 gene were subject to direct DNA sequencing analysis and mutation detection analysis (Surveyor mutation detection kit, Transgenomic, Elancourt, France). No mutations were found in the NPHS2 gene. However, the proband was found to have three heterozygous mutations in NPHS1: c.1096A>C (p.S366R) in exon9, c.649\_650delGT (p.Cys217fsX) in exon6 and c.791C>G (p.P264R) in exon7. The mother was found to be heterozygous for c.1096A>C (p.S366R) while the father was heterozygous for the novel c.649\_650delGT,

the known c.791C>G (p.P264R) and additionally a novel c.1619C>A (p.A540E) in exon12. In-silico analysis (SIFT, <http://blocks.fhcrc.org/sift/SIFT.html>, polyphen, <http://genetics.bwh.harvard.edu/pph>) indicated that p.A540E is not pathological, which is confirmed since the unaffected father also carries c.649\_650delGT (p.Cys217fsX) in exon6 and c.791C>G (p.P264R) in exon7. The novel 2bp deletion is predicted to cause a premature termination codon, and since it is apparently incis to the previously described c.791C>G mutation, presumably the deletion mutation has the over-riding impact on the expression of the phenotype.

## P05.029

## Creatine deficiency syndromes in Spain: enzymatic and molecular genetic studies

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Creatine deficiency syndromes (CDS) are a group of underdiagnosed inborn errors caused by defects in the biosynthesis of creatine or its transporter. The two defects affecting biosynthesis, namely arginino-glycine amidinotransferase (AGAT) and guanidinoacetate methyltransferase (GAMT) are autosomal recessive traits, whereas the creatine transporter (CRTR) is an X-linked disorder.

We present the results of a collaborative CDS prospective screening based on abnormal metabolite excretion or decreased creatine signal in brain MRS in a cohort of patients with mental retardation (MR) and/or suspected metabolic disorder. Three GAMT and nine CRTR cases were selected. Confirmatory diagnosis was assessed by measuring GAMT activity or creatine uptake in fibroblasts using MS/MS methods. Direct sequencing analysis of GAMT and SLC6A8 genes was carried out.

In two of the three potential GAMT patients, deficient activity was confirmed in fibroblasts (<4 % of matched control), and three new sequence variations were detected: c.145delT (p.Y49fs), which appeared in homozygous fashion, and c.316C>T (p.Q106X) and c.407C>T (p.T136M), both in heterozygous fashion. Four out of the nine CRTR cases selected (two from the same family) presented a deficient creatine transport in fibroblasts. Mutational analysis of SLC6A8 gene led us to identify three nucleotide changes, two of them novel: c.1210G>C (p.A404P); c.1079-1081delTCT (p.F360del). All changes identified in both genes fulfilled several criteria to be considered as pathogenic mutations.

Our results confirm the presence of CDS patients in a broad population of neurological patients including those with MR of unknown aetiology highlighting the importance of screening for these potentially treatable disorders.

## P05.030

## Two novel mutations in the CYP17A1 gene causing Congenital Adrenal Hyperplasia and severe Hypertension in a 46,XY Female Patient

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In the steroidogenic pathway, cholesterol is converted to pregnenolone which can be processed to either mineralocorticoids in adrenal glands or to sex steroids in the gonads. This effect is due to a 17α-hydroxylation and 17, 20-lyase activity respectively. The microsomal enzyme cytochrome 450c17 has a deep impact in the progress of the steroidogenic pathway because it has both 17α-hydroxilase and 17, 20-lyase enzymes activities. Mutations in the CYP17A1 gene cause 17α-hydroxilase deficiency (17OHD), an unusual form of Congenital Adrenal Hyperplasia (CAH). This anomaly occurs in 1/50000 newborns and it accounts for nearly 1% of the CAH cases. Classical adrenal and gonadal phenotype of the complete 17OHD includes hypertension and hypokalemia, secondary to massive overproduction of the 17-deoxysteroid, 11-deoxycorticosterone (DOC) and corticosterone in patients with a 46,XY female phenotype. Nevertheless, there is considerable variation in the clinical and biochemical features of 17OHD. Approx-

mately 56 mutations have been described in the *CYP17A1* gene. We present the molecular characterization of two novel mutations in this gene in a mexican-mestizo 46,XY female with CAH and severe hypertension who does not respond to treatment. Gene sequence revealed a compound heterozygous (K110X/ R362H) leading to a complete lack of enzyme activity. The patient was heterozygote for four SNPs. We suggest that these polymorphisms could be related to the failure of blood pressure treatment. We propose that all DSD patients require a multidisciplinary team to determine the etiology and orient the therapeutic approach to minimized medical, psychological and social complications.

#### P05.031

##### Molecular genetic analysis of CYP2D6 gene and used methods

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The cytochrome CYP2D6 is enzyme responsible for metabolism of many commonly used drugs such as tricyclic antidepressants, neuroleptics, beta blockers and antiarrhythmics. CYP2D6 is localized together with pseudogenes CYP2D7 and CYP2D8 at chromosome 22 and it is highly polymorphic. The polymorphisms leads to different individual responses following drug administration and increased risk of adverse reactions or the lack of the therapeutic response. According to the enzymatic activity the population has been grouped as - poor metabolizers, intermediate metabolizers, effective metabolizers, ultra-rapid metabolizers. There are studied influences of polymorphisms to the antidepressants treatment for a long time in Department of Medical Genetics and Department of Psychiatry.

Methods such as direct sequencing and agarose electroforesis are subsequently combinatied with more modern, faster and more effective method - Real-Time PCR and High Resolution Melting using the Light Cycler 480 System. Recently we can detect the most frequent null alleles 3\* 4\* 6\* 7\* and 8\* using the specific fluorescent labeled probes. Up to 99% of poor metabolizers in the Caucasian population can be detected with genetic testing for only 5 alleles (plus allele 5\*, which can be detected by using the Sybr green). The High Resolution Melting has been performed for the most frequent SNP's in CYP2D6 gene. Heterozygous samples are readily distinguished from homozygous and wild-type samples with used dyes. This paper provides an overview of current technologies available for assessing polymorphisms in Department of Medical Genetics.

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#### P05.032

##### The Clinical Use of Fluorescent Repeat-Primed PCR Assay in the Diagnosis of Myotonic Dystrophy Type 1

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Some trinucleotide repeat expansion disorders require Southern blot analysis to confirm the presence or absence of a large repeat expansion adding days to turnaround time. Cagnoli et al. (2004) successfully applied the use of fluorescent repeat-primed PCR (RPPCR) method to differentiate between patients who are homozygous for a normal-sized allele and those who are heterozygous for a very large expansion for Friedreich ataxia and spinocerebellar ataxia types 10 and 12. Using a slight modification of this RPPCR method, we were able to consistently detect the presence of large repeat expansions in the 3'UTR of the DMPK gene, the mutation underlying myotonic dystrophy type 1. To assess the validity of this assay in a clinical setting, all 63 patient samples and 9 quality assurance samples received over 18 months for myotonic dystrophy type 1 were analyzed by the usual "gold standard" method as well as by RPPCR. In the standard method, the number of CTG repeats is determined using fluorescent PCR followed by capillary electrophoresis. For a situation where only one normal-sized repeat is observed or two normal-sized repeats that are only one trinucleotide repeat apart in size, the sample is further tested by Southern blot analysis. A review of all data showed 100% correlation between the results obtained from the "gold standard" and RPPCR. As RPPCR can only show the presence of the expansion and cannot provide information

regarding size of the expanded allele, Southern blot must be pursued for RPPCR-positive patients. For RPPCR-negative patients, Southern blot analysis is unnecessary.

#### P05.033

##### Human miRNAs on chromosome 21 are differentially expressed in Down syndrome fetal hearts

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We previously demonstrated dosage-dependent upregulation of chromosome 21 (Hsa21) genes and dysregulation of mitochondrial and ECM genes in heart tissues of Down syndrome (DS) fetuses. Some of these dysregulated genes might be responsible for the DS cardiac phenotype, but it is evident that also other functional non-coding sequences, such as miRNAs, might play an important role. MiRNAs are highly expressed in the heart and regulate cardiac development and function. Five miRNAs, according to Sanger miRBase, are on Hsa21: miR-99a, miR-125b, let-7c, miR-155 and miR-802. Nothing is known about their expression in trisomic tissues.

We evaluated by qRT-PCR the expression of Hsa21 miRNAs in heart tissues from DS fetuses and controls. We found that miR-99a, miR-125b, let-7c and miR-155 were expressed in 20-22 weeks fetal heart. MiR-802 was not expressed. MiR-99a, miR-155 and let-7c were overexpressed in trisomic hearts, whereas miR-125b was normoregulated. Target genes of upregulated miRNAs were obtained by merging PicTar, TargetScan and MiRanda prediction lists. As miRNAs could affect protein expression by either interfering with RNA translation or promoting mRNA degradation, we evaluated the mRNA expression of target genes of overexpressed miRNAs by using the data set of our previous study. Seventeen targets of miR-99a, 12 of miR-155 and 15 of let-7c were expressed in fetal heart and downregulated in trisomic samples. Target genes possibly involved in DS phenotype were found such as SLC25A4, let-7c target, downregulated in DS hearts and involved in mitochondrial function, and CYP26B1, miR-99a target, showing a dosage-dependent effect on ventricular septal defects.

#### P05.034

##### Study of folate genes alteration CBS 844Ins68 and MTR A2756G as maternal risk factor of Down syndrome among Iranian cases

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Down syndrome is the most common chromosomal abnormality occurred 1 in 700 live birth. Abnormal segregation of chromosome 21 in meiosis is the cause of trisomy 21. Common polymorphisms in enzymes coding genes in folic acid pathway have been suggested to play role in etiology of chromosome 21 nondisjunction. In this study, the role for 844ins68 polymorphism in cystathione beta synthase (CBS) gene and A2756G polymorphism in methionine synthase gene (MTR) in folic acid pathway has been investigated as maternal genetic risk factor of Down syndrome among Iranian patients. CBS 844Ins68 polymorphism is a 68 bp insertion in exon 8 of this gene and MTR A2756G polymorphism causes an A>G transition at 2756 bp. We have studied 93 mothers having DS children and 116 aged matched control mothers for the frequency of above polymorphisms using RFLP PCR analysis. Genomic DNA was extracted from blood Leukocytes by salting out procedure. Frequencies of 844Ins68 polymorphism were 8 (8.6%) in mother cases and 18 (15.5%) in control mothers with no homozygosity in both groups. Frequency of A2756G polymorphism was observed as AA in 56 (60.2%) AG in 33 (35.5%) and GG in 4 (4.3%) in cases mothers and were 56%, 35.3% and 10% among control mothers respectively. Statistical analysis showed no association between 844ins68 and A2756G polymorphisms and risk of Down syndrome in Iranian mothers (P-value for 844Ins68 and A2756G were 0.146 and 0.451 respectively). Combination of these polymorphisms also have no effect on risk of Down syndrome's mother (P-Value was 0.216).

**P05.035****Supporting the appropriate ordering of genetic laboratory tests in the UK healthcare workforce****C. Barker, C. Bennett, C. Cooley, P. Farndon;**

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To address an increased need to promote the clinically appropriate and equitable ordering of genetic laboratory tests in the UK, the NHS National Genetics Education and Development Centre is working to raise understanding and knowledge of genetics in the UK healthcare workforce for non-genetics specialists.

The Centre identified the key knowledge and attitudes required to encourage appropriate ordering of genetic laboratory tests as part of a wider project to integrate genetic skills into clinical practice, job planning, evaluation, education and training. The knowledge and attitudes identified allow healthcare staff to recognise where genetic tests will inform clinical management within the limits of their role and where referral is appropriate. Healthcare staff will also be equipped to recognise any social, ethical or legal implications in ordering the genetic laboratory test.

To integrate these skills into clinical practice, the Centre has targeted health professionals for whom genetics is highly relevant and is working with these groups and other professional bodies to identify how genetics affects their clinical practice.

Using this information, the Centre is creating tools allowing healthcare staff to recognise where genetics impacts on their clinical practice. For example, relevant case scenarios allow healthcare staff to draw parallels with their own clinical practice. These tools are further supported by the Centre through the provision of education and training support and learning and teaching resources.

It is envisaged that this work will lead to more appropriate and equitable ordering of genetic tests and ultimately the enhancement of patient care.

**P05.036****Vascular type of Ehlers-Danlos syndrome: Evidences for a stochastic effect of COL3A1 haploinsufficiency****A. Plancke<sup>1</sup>, M. Holder-Espinasse<sup>2</sup>, V. Rigau<sup>3</sup>, C. Rene<sup>1</sup>, M. Taulan<sup>1</sup>, B. Catteau<sup>2</sup>, R. Steir<sup>2</sup>, S. Coopman<sup>2</sup>, N. Pallares-Ruiz<sup>1</sup>, S. Manouvrier-Hanu<sup>2</sup>, M. Claustres<sup>1</sup>, P. Khau Van Kien<sup>1</sup>;**<sup>1</sup>CHU Montpellier/INSERM U827, Montpellier, France, <sup>2</sup>CHRU de Lille, Lille, France, <sup>3</sup>CHU Montpellier, Montpellier, France.

Mutations that confer an unusual pattern of inheritance in a gene related to a well known genetic disease can sometimes highlight a particular mechanism useful to correlate genotype to phenotype. Here we describe a case of recessive Ehlers-Danlos syndrome (EDS) in a young girl of asymptomatic and related parents (uncle-niece). She exhibited: atrophic scars, extensive bruising, joint hypermobility and died at 12 years-old from an extreme intestinal fragility. According to the Villefranche nosology, she fulfilled the criteria of EDS vascular type for laboratory testing. Total sequencing of COL3A1 cDNA (obtained from skin cultured fibroblasts) identified an homozygous nucleotide duplication (c.479\_480dupT) resulting in a premature termination codon (p.Val160fsX46). Studies in genomic DNA showed that this mutation in exon 5 of the COL3A1 gene was inherited from each parent. As expected, the expression analysis (RT-PCR, quantitative-PCR, Immunohistochemistry, WB) showed a strong mRNA decay, which results in an absence of type III collagen in the proband.

This case, shows that a deficit in collagen III is viable in early childhood in Man. Here, the expected COL3A1 haploinsufficiency in her asymptomatic ascendants did not lead to the severe clinical manifestations of EDS vascular type caused by haploinsufficiency of one allele as described in the literature. This case provides evidences for a stochastic effect of COL3A1 haploinsufficiency in Man with (a) modifying factor(s), which remains to be identified.

**P05.037****Early myoclonic encephalopathy caused by a disruption of the Neuregulin-1 receptor ErbB4****H. Van Esch<sup>1</sup>, B. Ceulemans<sup>2</sup>, J. Vermeesch<sup>1</sup>, K. Devriendt<sup>1</sup>, L. Backx<sup>1</sup>;**<sup>1</sup>Center for Human Genetics, Leuven, Belgium, <sup>2</sup>Rehabilitation and Epilepsy Centre for Children and Youth, Pulderbos, Belgium.

The tyrosine kinase receptor ErbB4 (erythroblastic leukemia viral oncogene homolog 4) plays a crucial role in numerous neurobiological processes in the developing and adult brain. One of the most important and well-studied ligands of ErbB4 is Neuregulin-1 (NRG1) and it was shown that NRG1-ErbB4 signaling is essential for neurobiological processes like neurogenesis, migration, synaptic plasticity and differentiation of neurons and glia. Moreover, recent molecular genetics studies implicate ErbB4 in the pathophysiology of schizophrenia. However, the phenotypic consequences of haploinsufficiency of ErbB4 are not known, since no coding mutations have been identified until now. Here, we present a patient with early myoclonic encephalopathy and profound psychomotor delay with a *de novo* reciprocal translocation t(2;6)(q34;p25.3), disrupting the *ErbB4* gene. This patient represents the first case of haploinsufficiency for one of the ErbB family members of tyrosine kinase receptors.

**P05.038****Mutation analysis of epidermolysis bullosa in the Czech Republic****B. Jerabkova<sup>1,2</sup>, L. Fajkusova<sup>1,2</sup>, H. Buckova<sup>3,4</sup>, K. Vesely<sup>5,6</sup>, R. Gaillyova<sup>7,2</sup>;**<sup>1</sup>Centre of Molecular Biology and Gene Therapy, Faculty Hospital Brno, Brno, Czech Republic, <sup>2</sup>Masaryk University Brno, Brno, Czech Republic, <sup>3</sup>Department of Pediatric Dermatology of 1st Pediatric Clinic, Faculty Hospital Brno, Brno, Czech Republic, <sup>4</sup>Institute of Medical Postgraduate Studies Prague, Prague, Czech Republic, <sup>5</sup>1st Institute of Pathological Anatomy, St. Ann's Hospital, Brno, Czech Republic, <sup>6</sup>Medical Faculty of Masaryk University Brno, Brno, Czech Republic, <sup>7</sup>Department of Clinical Genetics, Faculty Hospital Brno, Brno, Czech Republic.

Epidermolysis bullosa (EB) is a clinically and genetically heterogeneous group of heritable skin disorders. It is characterised by skin blistering and mucous membrane. EB has been divided into three major categories based on the level of blister formation in dermal-epidermal junction zone.

EB is diagnosed by evaluation of clinical findings, by transmission electron microscopic examination of a skin biopsy and immunohistochemical mapping of protein components of dermal-epidermal junction zone from a skin biopsy. Molecular-genetic diagnostics of EB was initiated in 2004.

Dystrophic EB (EBD) is caused by mutations in the collagen type VII (COL7A1), which consists of 118 exons. EB simplex (EBS) is caused by mutations in the keratin 5 (KRT5), which consists of 9 exons and keratin 14 (KRT14), which consists of 8 exons.

DNA from EB patients and their relatives were screened for mutations in COL7A1, KRT5 and KRT14 genes. Analysis was performed using PCR, denaturing high performance liquid chromatography, high resolution melting analysis and direct sequencing. We could identify KRT5 or KRT14 dominant mutations in 11 out of 18 EBS families. As regards 27 EBD probands, we revealed disease causative mutations in 16 patients and screening of COL7A1 is in progress. Prenatal diagnosis of one pregnancy in family with occurrence of EBS predicted the fetus being normal and subsequently a healthy child was born.

Determination of EB at the level of DNA has important implication for final confirmation of diagnosis, possibility of genetic counselling and early prenatal diagnosis.

This work was supported by IGA MH NR9346-3.

**P05.039****Erythropoietic protoporphyria in a Czech family caused by a new 84G>A (W28X) mutation in the ferrochelatase gene****J. Prochazkova<sup>1</sup>, J. Sperl<sup>2</sup>, S. M. Farrag<sup>1</sup>, L. Barnincova<sup>1</sup>, J. Spicak<sup>2</sup>, P. Matasek<sup>1</sup>;**<sup>1</sup>1st School of Medicine, Charles University, Prague, Czech Republic, <sup>2</sup>Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

Erythropoietic protoporphyria (EPP) is a disorder with autosomal dominant inheritance caused by partial deficiency of ferrochelatase (FECH). Ferrochelatase is the ultimate enzyme of heme biosynthesis. EPP is characterized by excess accumulation of protoporphyrin, particularly

in the erythroid cells of the bone marrow. Biochemical features of EPP include increased protoporphyrin levels in erythrocytes, plasma, feces, and bile. Clinical manifestations of the disease are characterized by cutaneous photosensitivity, which almost always appears in childhood and includes burning, itching, swelling, and redness in sun-exposed areas. Hepatic failure occurs in some patients (about 1-10% of EPP patients) which may necessitate liver transplantation. The gene encoding human FECH is localized on chromosome 18q21.3, and spans over 45kb with eleven exons. The cDNA encodes a protein containing 423 amino acids, and the enzyme exists as a homodimer of 86 kDa. Each subunit contains residues 65-423 and one [2Fe-2S] cluster. Mutational analyses of the FECH gene revealed a novel unpublished mutation in the FECH gene in a patient from Czech Republic with EPP: a G→A transition at position 84 in exon 2. This point mutation alters to a tryptophan to a stop codon (W28X). The amino acid tryptophan at position 28 is located in a mitochondrial targeting sequence spanning amino acid residues 1-62 that is removed during proteolytic processing. Analyses were carried out on seven members of proband's family; all persons, except one, are asymptomatic carriers.

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#### P05.040

##### Identification of the molecular defects in Turkish FHL patients assigned to Perforin and Munc13-4 genes

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Familial Hemophagocytic Lymphohistiocytosis (FHL) is a rare autosomal recessive, if untreated, fatal disorder of early childhood. The purpose of this study is to evaluate the genetic defects underlying clinical phenotypes observed in Turkish patients with this disorder. The subjects of this study were a total of 79 FHL patients (52M/27F) from 74 unrelated families (consanguinity:62, family history:31). Linkage analysis used for subtyping the patients to FHL type II revealed homozygosity or consanguineous common alleles in 19 of these families. Direct sequencing of Perforin gene led to the identification of 5 different sequence changes in 12 families. Six patients had nonsense W374X mutation in homozygous state except one who was coming from a non-consanguineous family. Two patients had G149S, one V50M, two A91V and one novel A523D homozygous missense changes. Mutations in this gene account for about 16% of Turkish FHL patients in this study. On the other hand, 20 families were found to show either homozygosity or consanguineous common alleles for Munc13-4 gene in the linkage analysis. These patients were screened for mutations in 32 exons of Munc13-4 gene by SSCP/heteroduplex analysis. Sequencing the aberrant bands, thus far, led to the identification of 3 different homozygous mutations in 3 unrelated patients who were coming from consanguineous families. A frameshift (627delT) mutation was detected in a female patient who also had heterozygous A91V mutation in Perforin gene while nonsense (R1065X) and novel missense (R414C) mutations were found in 2 male patients. This study was supported by TUBITAK (Project No: 105S386; SBAG-3193).

#### P05.041

##### Six new mutations in *UNC13D* gene in Russian patients with familial hemophagocytic lymphohistiocytosis (FHL)

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FHL is a rare autosomal recessive disorder of immune regulatory pathways characterized by a defect in natural killer cell function. *UNC13D* gene codes a protein involved in vesicle priming function and its mutations have been shown to cause FHL. We have examined five Russian unrelated patients with FHL in age ranged from 1 to 5 years (two familial and three sporadic cases). We have investigated DNA samples for mutation in *UNC13D* gene coding area by direct sequencing. All our patients were found to be compound heterozygotes, carrying two mutations (c.2343 del 2344-2347 / c.3037 ins G; c.3037 ins G / c.3173 T>C (p.1058 Leu > Pro); c.627 del T / c.1828 ins A; c.322-1 G>A (CD 042833) / c.3037 ins G; c.2215 del 2216-2239 / c.2343 del 2344-2347). Only one mutation c.322-1 G>A (CD 042833) has been previously reported, and six others are new. The mutation c.2343 del 2344-2347

has been observed in two chromosomes and c.3037 ins G in three chromosomes. According to our results we propose that there might be "hot points" in *UNC13D* gene, where mutations are found with higher frequency.

#### P05.042

##### Familial hypercholesterolemia: experience from a portuguese genetic department

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Familial Hypercholesterolemia (FH) is an autosomal dominant disorder, usually caused by mutations on the low density lipoprotein receptor gene (LDLR), the gene encoding apolipoprotein B (APOB) or *Proprotein Convertase Subtilisin/Kexin type 9* (PCSK9) which is associated with premature atherosclerosis and coronary heart disease (CHD).

**OBJECTIVES:** Clinical and molecular characterization of patients and their children with family history of premature CHD and abnormal lipid profile.

**MATERIAL AND METHODS:** a clinical questionnaire from the "Portuguese FH Study" was completed, characterized their lipid profile and study mutations in LDLR, APOB and PCSK9 genes.

**RESULTS:** We observed 163 persons, 111 adults and 52 children in 56 families. 13 patients with CHD were younger than 50 years of age. The mean total cholesterol was 264, 41 mg/dL and 294,09 mg/dL and mean LDL-cholesterol was 189,87 mg/dL for and 206,1 mg/dL for children and adults respectively. All families received counseling regarding lifestyle and dietary modifications. After dietary or pharmacological therapy, mean cholesterol was 222,14 mg/dL and 238,6 mg/dL and mean LDL-cholesterol was 148,25 mg/dL and 160,6 mg/dL for children and adults respectively. Molecular study of the LDLR gene was performed in 56 families, and a mutation was found in 23 families. No mutations were identified in APOB or PCSK9 genes.

**CONCLUSION:** Molecular study of FH patients offers the possibility of identification of the mutation in relatives at risk for premature atherosclerosis. Early diagnosis allows earlier lifestyle modifications and dietary or pharmacological intervention in mortality and morbidity.

#### P05.043

##### Functional analysis of potential splice site mutations by RT-PCR of LDLR mRNA isolated from fresh blood mononuclear cells

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder associated with high risk of coronary heart disease. FH is caused mainly by inherited defects on the Low Density Lipoprotein Receptor gene (LDLR) resulting in increased circulating LDL cholesterol. Of the many different LDLR mutations found in FH patients worldwide, about 6 % of single base substitutions are located near or within introns and are predicted to result in exon skipping, retention of an intron or activation of cryptic sites during mRNA splicing. Ten such mutations, four of them novel, were found in the "Portuguese FH Study", and those whose effect on splicing was untested have been investigated by RT-PCR of LDLR mRNA isolated from fresh blood mononuclear cells. Four of these variants (313+6 T>C, 1060+1G>A, c.2389G>T (p.V776L), 2547+1G>A) caused exon skipping, and one caused retention of an intron (c.1359-5C>G), while two others (c.2140+5 G>A and c.1061-8T>C) had no apparent effect. Variants in two patients lost to follow-up could not be tested experimentally, but they almost certainly affect splicing because they disrupt the invariant AG or GT in acceptor (818-2A>G) or donor (1845+1delG) splice sites. These mutations rep-

resent 9% of all mutations in the "Portuguese FH Study". Our findings emphasize that care must be taken before reporting the presence or absence of a splice site mutation in *LDLR* for diagnostic purposes. Our study also demonstrates that relatively simple, quick and inexpensive RNA studies can evaluate putative splicing mutations that are not always predictable by available software, thereby reducing genetic misdiagnosis of FH patients.

#### P05.044

##### **Co-occurrence of four mutations in a clinical case of Familial Mediterranean Fever**

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Familial Mediterranean Fever (FMF) type 1 is characterized by recurrent short episodes of fever associated with different inflammatory processes. MEFV is the only gene currently known to be associated to FMF. E148Q in exon 2 and several mutations in the exon 10 of MEFV are the most common. FMF is inherited in an autosomal recessive manner, so both parents of a proband are considered obligate carriers. We studied a FMF clinical case and his family and surprisingly four different mutations were found.

The family consisted in the affected proband, a sister and the parents, all of them unaffected. The study was carried out by bidireccional sequencing of exon 10 and targeted mutation analysis by RFLPs of E148Q mutation in exon 2 of MEFV gene.

The E148Q was present in heterozygous state in the proband. The sequencing analysis of the exon 10 showed the p.R653H and p.I640M mutations and the deletion p.I692del, all of them previously described. The study of familial samples revealed the presence of two mutations in each and everyone: father p.I640M and p.R653H, mother E148Q and p.I692del and sister p.I640M and p.R653H (haplotype in *cis* phase from his father).

The inheritance pattern showed that each parent carries two mutations in *cis* phase, the sister inherited the wild type phase of the mother and the mutated phase of the father and the affected son is therefore carrying all four mutations.

This case makes us aware of the importance of considering the haplotype phase of multiple mutations in recessive disorders.

#### P05.045

##### **MEFV Mutations in Turkish Patients Suffering From Familial Mediterranean Fever**

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Familial Mediterranean fever (FMF) is an autosomal recessive periodic disorder. Over 50 mutations have been identified in the MEFV gene responsible for FMF. For diagnosis of FMF; molecular methods and Tel-Hashomer Clinical Criterias (THCC) can be used. AIM: To identify distribution and frequency of the MEFV gene mutations in Turkish FMF patients, compare two molecular different technics and correlation of clinical-molecular diagnosis. PATIENTS-METHODS: The study was carried out on 604 clinically diagnosed Turkish FMF patients. Mutation screening of the MEFV gene was performed by sequencing of exon 10 in 448 patients and by FMF specific StripAssay (PCR-Reverse Hybridization) for E148Q, P369S and F479L mutations of exons 2,3, and 5, respectively in 256 patients. RESULTS: Of the 604 unrelated patients investigated, 344 (56.95%) had one or two mutations : 85 patients (24.71%) were homozygous; 88 (25.58%) were compound-heterozygous; 171 (49.71%) were heterozygote mutations. Of the mutations, M694V (A>G), V726A, M680I (G>C), R761H accounted for 65.99, 19.18, 17.44, and 4.36 %, respectively. StripAssay was observed completely correlate with direct sequencing in 100 patients. For determining the correlation between mutation status, clinical diagnosis with THCC was investigated in 90 patients. Only 33 patients were exactly diagnosed by THCC whereas 53 patients were reported mutations by StripAssay. CONCLUSION: Exon 10 is the most common site for FMF mutations whereas exons 2,3 and 5 accounts for 11.63% of the cases. The most common mutation among Turks is M694V (A>G). As a result,

StripAssay for 12 common mutations might be used in routine mutation screening analysis.

#### P05.046

##### **Promoter studies in the FA genes**

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Fanconi anemia (FA) is a rare genome-instability disorder with the frequent presence of congenital malformations and bone marrow failure. Other characteristic features include predisposition to FA-typical malignancies and cellular hypersensitivity to DNA-interstrand crosslinking. At least 13 genes and corresponding complementation groups are underlying the disease. Eight of the FA proteins (FANCA, -B, -C, -E, -F, -G, -L and -M) and other components assemble in a nuclear complex, the FA "core complex".

Little if any is known about the promoter regions. However, identification and characterization of the promoters would be essential for understanding the regulation of transcription, including intergenic regulation, of the FA genes. Our aim is to provide an explanation for the equimolar composition of the core complex. We used a variety of *in silico* methods to predict potential promoter regions. To confirm these, we have set up dual luciferase reporter assays. We cloned the identified regions (~1kb) in the pGL3 basic vector that carries the reporter gene for firefly luciferase. As co-reporter we used the pRL null vector that contains the renilla luciferase gene. With these constructs, we transfected HeLa and HEK293 cells to assay luciferase activity using a luminometer. In a second approach we used mutated constructs to detect any decrease of activity. Further characterization includes transcription factor binding sites and conserved sequence elements. First results indicate that there is generally strong promoter activity. Short half-life of the gene products is consistent with high transcriptional activity of the FA genes, and rapid regulation in response to DNA damage.

#### P05.047

##### **MYCN gene mutation screening in 9 patients with Feingold syndrome**

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MYCN is a transcription factor amplified in about 30% of neuroblastoma. MYCN is also the disease-causing gene in Feingold syndrome, with a dominant mode of inheritance. Feingold syndrome is defined by microcephaly, digital anomalies (syndactyly, brachymesophalangy), digestive atresia (oesophagus, duodenum), facial features (retrognathia, small palpebral fissures) and occasional malformations of heart, kidney and spleen.

We studied the coding sequence of MYCN in 9 patients and found 3 mutations in the bHLH or the Leucine Zipper domain: 2 frameshift mutations leading to a truncated protein and a nonsense mutation. Loss-of-function is highly likely for frameshift mutations, while a dominant-negative due to altered DNA binding and preserved dimerisation is hypothesised for the nonsense mutation. MYCN deletion are currently being tested for the remaining patients.

We performed *in situ* hybridization on human embryos (Carnegie stages 12, 15 and 17) to determine MYCN expression pattern during development. At C12, MYCN is ubiquitous expressed. Later (C15 and C17), expression is restricted to mesencephalon, diencephalon, spinal cord, limb buds, oesophagus and stomach, in accordance with Feingold syndrome features. MYCN is also expressed in the primitive mesonephros and the Rathke pouch. These later findings argue for kidney anomalies to be regarded as a feature of Feingold syndrome and raise the hypothesis of an endocrine involvement for the short stature observed in the syndrome.

#### P05.048

##### **Molecular characterization of familial hypercholesterolemia in Iranian patients**

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Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused mainly by mutations in the low-density lipoprotein receptor (LDLR) and apolipoprotein B (APOB). Until now, the molecular basis of FH has been demonstrated in detail in many populations, but there is still very limited Molecular data concerning FH in Iran. The aim of this study was to characterize the LDLR and APOB gene mutations in an Iranian population.

A total of 50 non-related Iranian heterozygous FH subjects were studied. All samples were initially tested for 3 common APOB gene mutations including R3500Q, R3500W and R3531C using PCR-RFLP assay. Subsequently, LDLR gene were screened partially (exons 2, 4, 6, 7, 8, 9, 10, 12 and 14) by PCR-SSCP analysis and positive results were confirmed by DNA sequencing.

Four previously reported polymorphisms 1413 G >A, 1725 C >T, 1773 T > C and 2140 +5G>A were found in 18% of population studied. Moreover, no variation was found in APOB gene. Our data indicated that LDLR and APOB gene mutations have not contribution to FH in Iranian population studied here. However, we examined 3 common APOB mutations and 9 exons of LDLR in only 50 patients, and to determine the role of these genes in developing FH in Iran, more samples/populations needed to be investigated for the whole coding regions and promoter of the genes.

#### P05.049

#### Correlation of MEFV gene mutations and bone mineral density in children with familial mediterranean fever

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**Objective:** Familial Mediterranean Fever (FMF) is an inherited disorder caused by an abnormal recessive gene. Virtually all cases are due to a mutation in the MEFV gene, which codes for a protein called pyrin or marenostenin. The aim of this study is evaluating the correlation between bone mineral density and mutations in children with FMF.

**Methods:** 36 prepupal children diagnosed FMF according to Tel Hashomer Criteria were included in the study. Bone mineral density (BMD) was measured in all patients by dual energy X-ray absorptiometry, both at the lumbar spine (antero-posterior projection of L1-L4) and total body. BMD data were expressed as grams per centimeter square and standard deviation scores (Z score). The five MEFV gene mutations (M694V, M694I, V726A, M680I, E148Q) were scanned in all cases by PCR- ELISA method. According to the results of the genetic investigation, cases were grouped as cases with no scanned mutations and cases with heterozygous and homozygous mutations.

**Results:** Both lumbar vertebrae and total body bone mineral density of the patients were found to be low. No differences were detected among BMD values of the groups.

**Conclusion:** In conclusion, no significant relation was detected between MEFV gene mutations and BMD in the patients with FMF. This result does not mean there is no low bone density risk in patients with FMF. Due to this, we think that study will be significant with more patients and in association with biochemical markers, signs of bone health.

#### P05.050

#### The prevalence of familial Mediterranean fever gene mutations in patients with rheumatic heart disease

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**BACKGROUND:** Acute rheumatic fever (ARF) has been considered in the differential diagnosis of familial Mediterranean fever (FMF) because these two diseases have some clinical and laboratory similarities. There are also autopsy reports of rheumatic mitral stenosis in patients with FMF and amyloidosis. Moreover, a history of ARF during childhood is not infrequent in patients with FMF.

**OBJECTIVE:** To investigate the prevalence of familial Mediterranean fever gene mutations in patients with rheumatic heart disease.

**METHODS:** A total of 21 patients with rheumatic heart disease were enrolled. The diagnosis of mitral stenosis was established with echocardiography or angiography. Patients with predominant mitral regurgitation or isolated aortic or tricuspid valve disease were excluded. Genetic analysis was carried out by the NanoChip® Molecular Genetics Workstation.

**RESULTS:** Four of the patients were found to have heterozygote MEFV mutations. Three of these mutations were E148Q- and one was V726A-.

**CONCLUSION:** In the light of our preliminary results, we may conclude that the frequency of MEFV mutations are not higher than the normal population. Further studies with larger sample sizes are needed for better understanding the possible relationship between these two disorders and to clarify whether specific mutations play role in the pathogenesis.

#### P05.051

#### Comparison of mutations screening assay on MEFV gene in Turkish FMF patients.

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Familial Mediterranean fever (FMF) is an autosomal-recessive disorder characterized by recurrent attacks of fever and serositis common in eastern Mediterranean populations. Over 70 mutations have been identified in the MEFV gene responsible for FMF. The aim of this study is to determine the frequency of the mutations which has been reported comparatively rare, to define the most effective mutation set, and to select the most suitable DNA analysis system for Turkish FMF patients.

1709 patients were referred by specialists to the Molecular Genetic Diagnostic Centrum of Duzen Laboratuaries Groups from various regions of Turkey. First, mutation screening of the MEFV gene was performed for the 3 most common mutations, namely M694V, M680I, V726A, in 1182 unrelated patients by polymerase chain reaction and restriction enzyme digestion analysis. The rate of mutation detection was 46.2% and these three mutations accounted for 64.4%, 22.6% and 12.7% of the alleles, respectively.. Second, we investigated 12 mutations (E148Q, P369S, F479L, M680I G>C, M680 G>A, 1692del, M694V, M694I, K695R, V726A, A744S and R761H) in 527 patients using reverse dot-blot hybridization (RDBH) assay. We found the rate of detection to be 50.5%. The most common mutations were found to be M694V, E148Q, M680I, V726A and R761H. Percengate of these mutations were 47.7, 19.9, 14.3, 9.8 and 4.1, respectively. Our study showed that the RDBH method increased the detection rate of FMF mutations about 4% compared to PCR-RFLP method for Turkish population.

#### P05.052

#### Missense mutations in the forkhead domain of FOXL2 lead to subcellular mislocalization, protein aggregation and impaired transactivation.

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Mutations of FOXL2, encoding a forkhead transcription factor, have been shown to cause blepharophimosis syndrome (BPES), characterized by an eyelid malformation variably associated with premature ovarian failure. Recently, polyalanine expansions and truncating mutations were shown to lead to protein mislocalization, aggregation and altered transactivation. Here, we study the molecular consequences of 17 naturally occurring FOXL2 missense mutations on subcellular localization and transactivation capacities in cellular systems. Most of these mutations map to the conserved DNA-binding forkhead domain (FHD). According to their subcellular localization in COS-7 cells, the mutant proteins could be divided into four groups. We also studied the trans-

activation capacity of the mutants in FOXL2 expressing granulosa-like cells (KGN). Several mutants led to a loss-of-function, while others might induce a dominant negative effect. Interestingly, one mutant that is located outside the FHD (S217F), proved to be hypermorphic and to have no effect on intracellular protein distribution. Clinically this mutation gives rise to a mild BPES phenotype, and to growth hormone deficiency. In general, missense mutations located in the FHD lead to a classical BPES phenotype, but cannot be correlated with the presence of an ovarian phenotype. However, a potential predictive value of localization and transactivation assays in the making of genotype-phenotype correlations is proposed. In conclusion, this is the first study to demonstrate that a significant number of missense mutations in the FHD of FOXL2 lead to mislocalization, protein aggregation and altered transactivation, and to provide insights into the pathogenesis associated with missense mutations of *FOXL2* in human disease.

#### P05.053

#### The spectrum of GALT gene mutations in south regions of Russia

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Galactose-1-phosphate uridylyltransferase (GALT) deficiency galactosemia is clinically heterogeneous autosomal recessive disorder. Newborn screening can identify patients with GALT deficiency galactosemia. The diagnosis needs to be confirmed by enzyme activity test. Unfortunately, in many cases the results of GALT activity measurement can be ambiguous and further molecular testing is required. More than 200 point mutations were revealed in the GALT gene, but the prevalence of these mutations varies among ethnic groups. Classical galactosemia newborn screening was started in Russia during 2006/2007 years. Total of 160 990 newborns were screened in South Russia and 11 patients with galactose level more than 7,2 mg/dL were revealed. Blood samples of this patients were submitted for confirmatory testing for classical galactosemia. The GALT gene were sequenced in all cases. The mutational spectrum included five missense mutations M142L, H186Y, Q188R, K285 N, N314D. The classical galactosemia was revealed in 4 cases with genotypes Q188R / Q188R, K285 N / M142L, H186Y / N, K285N / N; Duarte variant was revealed in 5 cases with genotypes Q188R / N314D, Q188R / N314D, , N314D / N314D, N314D / N, N314D / N. In 2 cases mutations were not founded

#### P05.054

#### Novel mutations in the gap junction gene GJB2 show that keratoderma is associated with connexin protein transport defects

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Mutations in the skin expressed gap junction gene GJB2 (coding for connexin26) cause a plethora of sometimes severe skin disorders with sensory deafness. Specific mutations are associated with distinct phenotypes and the reasons for this strong genotype-phenotype correlation are poorly understood. Commonly used functional parameters of gap junction functionality, such as dye transfer and electrical conductance, do not correlate with disease phenotype or severity. We have now identified a number of novel mutations that are specifically associated with palmoplantar keratoderma. Using fluorescent fusion proteins, we show that this skin symptom may be specifically caused by protein transport defects. What's more, its severity is inversely correlated to that of the transport defect. We are now working to understand the cellular sequelae of the disturbed protein trafficking. Preliminary data indicate that ER stress responses may be involved.

#### P05.055

#### Analysis of GJB2 gene exon 2 in Latvian patients with nonsyndromic sensorineural hearing loss

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Background: Nonsyndromic hearing impairment (NSHI) is the most common form of deafness. Mutations in the *GJB2* gene, which encodes gap-junction beta-2 protein (connexin 26), are the main cause

of recessive NSHI. It has been identified that one particular *GJB2* mutation named 35delG is the most prevalent for the populations of the European origin.

Materials: We obtained DNA samples from patients with prelingual NSHI 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* gene exon 2 analysis was performed using PCR, enzymatic restriction and automated sequencing.

Results: 55 unrelated patients were screened for the *GJB2* mutations. Mutations were detected in 67 of 110 (61%) tested alleles. Four different mutations in the *GJB2* gene have been identified in Latvian patients with NSHI: 35delG, 311-324del14, 235delC and M34T. 28 patients (51%) are homozygous for 35delG mutation, four patients (7%) are compound heterozygotes for 35delG and 311-324del14 mutations, one patient (2%) has genotype 35delG/235delC and one patient (2%) is heterozygous for M34T mutation. One heterozygous 51del12insA mutation was detected in unaffected individual with positive family history.

Conclusion: Our results verify the *GJB2* mutations to be causative for NSHL and confirm previous reports on the mutation distribution. Still, the cause of hearing loss remains unclear for patients with no or single *GJB2* mutation. However, *GJB2* related diagnosis cannot be excluded until mutations in non-coding regions and adjacent *GJB6* gene have been screened.

#### P05.056

#### Role of CYP1B1 mutations in Primary Open-Angle Glaucoma

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Glaucoma is a complex and genetically heterogeneous disease characterized by the progressive apoptotic death of retinal ganglion cells. Primary open-angle glaucoma (POAG) is the most common form of glaucoma, featured by an adult onset (>40 years), a gonioscopically open angle, and a reduced outflow facility. Heterozygous mutations in *CYP1B1* gene are presented in the 4-9% POAG patients of different populations.

Our purpose is to establish the genotype-phenotype relationship in Spanish POAG patients carrying *CYP1B1* mutations. We have analyzed the enzymatic activity of different *CYP1B1* mutations found in these patients, in transfected HEK-293T cells. The *CYP1B1* enzymatic activity assay was carried out using ethoxresorufin as a substrate in a fluorimetric assay.

*CYP1B1* mutations found in POAG patients show reduced enzymatic activity, supporting that loss of function mutations may play a role in the development of POAG.

#### P05.057

#### Analysis of secretion and processing of wild type myocilin and myocilin-glaucoma mutants co-expressed in cell lines

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Glaucoma is a leading cause of irreversible blindness worldwide. The main known risk factor for this disease is an elevated intraocular pressure (IOP), mainly caused by an increased resistance to aqueous humour outflow. Heterozygous mutations in the olfactomedin-like domain of the myocilin gene (*MYOC*) cause autosomal dominant juvenile-onset glaucoma, and approximately 4% of all adult-onset primary open-angle glaucoma (POAG) cases. The mechanisms by which these mutations elevate IOP and cause glaucoma are currently controversial. It has been described that myocilin undergoes an intracellular endoproteolytic processing by calpain II, in the middle of the polypeptide chain, which is reduced by glaucoma-associated *MYOC* mutations.

To gain insight into the molecular mechanisms by which mutations in the *MYOC* gene lead to glaucoma, we have analyzed, by SDS-PAGE

and Western blot, the secretion, endoproteolytic processing and extracellular aggregates of wild-type myocilin and 5 glaucoma-associated myocilin mutants (E323K, R346T, Q368X, P370L and D380A) transiently co-expressed in a cell line in culture. Our results show that coexpression affects secretion and proteolytic processing of wild type and mutant myocilins. These phenomena could play a role in the development of glaucoma.

#### P05.058

#### Screening for glucocorticoid-remediable aldosteronism among hypertensive patients in Bulgaria

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**Background.** Primary aldosteronism (PA) is currently considered the most frequent form of endocrine hypertension. Glucocorticoid-remediable aldosteronism (GRA) is a genetic variety of PA, affecting about 1% of patients, which is inherited in an autosomal dominant pattern. GRA is caused by a chimaeric gene with aldosterone synthase activity originating from an unequal crossing-over between the CYP11B1 (11 beta-hydroxylase) and CYP11B2 (aldosterone synthase) genes. Hypertension in GRA can be severe leading to cerebrovascular complications at a young age and female carriers of the mutation have a higher incidence of preeclampsia during pregnancy. On the other hand GRA can be successfully treated by glucocorticoids, which justifies its screening and early diagnosis. **Objective.** The aim of this study was to assess the prevalence of GRA among patients with confirmed PA. **Methods.** The study population consisted of 170 hypertensive patients, referred to a specialized Endocrinology department. In order to identify patients with PA we used the aldosterone to renin ratio and the Captopril test. The diagnosis of PA was confirmed in 11 subjects who were investigated for GRA using the long PCR technique. **Results.** None of our patients was positive for the CYP11B1/CYP11B2 chimaeric gene. **Conclusions.** Our study demonstrated that GRA can be successfully excluded in patients with PA, using the long PCR technique. Further studies in larger groups of patients are needed to evaluate the prevalence of GRA among patients with PA, which is probably lower than previously reported

#### P05.059

#### The *crv4* mouse model reveals a new role of the metabotropic glutamate receptor type 1 (*Grm1*) in the kidney glomerulus

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We recently described the *crv4* mouse mutant, where a spontaneous mutation causes the lack of the metabotropic glutamate receptor type 1 (*Grm1*). Homozygous *crv4* mice exhibit a complex phenotype, mainly characterized by ataxia and by morphological and functional renal anomalies.

By PCR screening of a cDNA library of human tissues, we found *Grm1* expressed in the kidney. Expression of *Grm1* was also evidenced by amplifying and sequencing cDNA obtained from renal tissues of wild type mice. The expression of the receptor was confirmed in wild type renal tissues by western-blot and immunofluorescence analyses, using specific antibodies.

Electron microscopy analyses of the *crv4* kidneys, compared to the wild type, evidenced major glomerular alterations of the glomerular basement membrane and the podocytes, and immunofluorescence analyses of specific podocyte proteins, such as nephrin, synaptopodin, and ZO1, showed a reduced expression in the mutated mice.

Urine analyses by ELISA showed a statistically significant albuminuria in *crv4* homozygous relative to wild type mice.

These evidences support a recent hypothesis according to which podocytes communicate by neuron-like mechanisms. The podocyte, similarly to the neuronal cell, has vesicular structures for the exocy-

tosis/endocytosis and release of glutamate. In this view, mutations of molecules involved in such mechanisms may be added to other known genetic causes of glomerulopathies.

#### P05.060

#### Identification, characterization and regulatory role of a new TFII-I family member, GTF2IRD2, located at the Williams-Beuren Syndrome locus

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*GTF2I*, *GTF2IRD1* and *GTF2IRD2* are three related genes located in the 7q11.23 Williams-Beuren Syndrome (WBS) locus that encode different members of the TFII-I family of transcription factors, characterized by the presence of several HLH-like domains known as I-repeats. The functions of *GTF2I* and *GTF2IRD1*, hemizygously deleted in all typical WBS patients, have been studied in depth. However, little is known about *GTF2IRD2*, a multicopy gene that is variably deleted in WBS patients, and thus a candidate to influence the variable severity of the phenotype. We have studied the function of the three expressed *GTF2IRD2* copies termed medial, telomeric and the chimeric found in some WBS patients. *In vitro* transfection assays in COS7 cells revealed that all three *GTF2IRD2* proteins formed heterodimers with *GTF2I*, but only the *GTF2IRD2-tel* copy could interact with *GTF2IRD1*. The cellular localization pattern was different among the three proteins. *GTF2IRD2-tel* protein appears mainly nuclear while the *GTF2IRD2-chi* was found to be mostly cytoplasmatic. The *GTF2IRD2-tel* and *GTF2IRD2-med* proteins, but not the *GTF2IRD2-chi*, changed their distribution pattern when co-transfected with *GTF2I* and *GTF2IRD1*. In addition, the *GTF2IRD2-tel* protein was able to activate transcription of the *c-fos* gene in a synergistic way with *GTF2I*. In conclusion, *GTF2IRD2* proteins appear to act as transcription regulators by virtue of interacting with *GTF2I* and *GTF2IRD1* with different functional outcomes. The variable amount of the different TFII-I family proteins in WBS patients, depending on deletion breakpoints, could contribute to modulate the variable expression of target genes and thus the WBS phenotype.

#### P05.061

#### Investigating Factor V (G1691A), Prothrombin (G20210A) and Methylenetetrahydrofolate reductase (C677T) gene polymorphisms in recurrent pregnancy loss

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In 75% of women trying to be pregnant, early pregnancy loss occurs. Habitual abortion is the termination of two or more consecutive pregnancies before 20th gestational week.

Various etiologic factors are responsible for recurrent pregnancy loss. In the performed studies these factors are reported to be, 7% chromosomal abnormalities, 10% anatomic problems, 15% hormonal irregularities, 6% unclear reasons and 55-62% coagulation protein/platelet problems. The importance of genetic defects causing deficiency in the coagulation system are better understood recently. Among them the most frequently related ones are some of the mutations take place in the Factor V, Prothrombin and the MTHFR genes.

In our study we objected to investigate the existence of the FV Leiden, Prothrombin and MTHFR gene mutations in 110 women with recurrent pregnancy loss and in 30 women with healthy children and no pregnancy loss. Mutation screening was performed by PCR-RFLP method using Hind III and Hinf I restriction enzymes for the blood samples of which DNAs were isolated.

FV Leiden, Prothrombin, MTHFR mutations were detected to be 13.6 %, 6.4%, 55.5% in the case and 6.7%, 6.7%, 53.3% in the control groups, respectively. No significant differences were detected between the case and the control group according to the mutation frequencies. It is thought that the risk of pregnancy loss is related to the combined augmentation of the thrombophilic mutations rather than a specific mutation. Probably, investigating prevalence of more thrombophilic mutations in women with habitual abortion will be more significant.

**P05.062****Duplication of exons 1 to 22 of the F8 gene: a new mechanism related to multiple copies of the intron 22 int22h sequence?**

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Interspersed repeats account for 56% of the euchromatic X chromosome sequence, compared with a genome average of 45%. Intrachromosomal duplications are estimated to account for 2.59% of the X chromosome while interchromosomal duplications account for a very small fraction 0.24% of the X chromosome. Among these duplications are well-described cases that are associated with genomic disorders. Some disorders may result from rearrangements involving duplicated sequences in Xq28, such as in haemophilia A. In severe haemophilia A, mutations are frequently the results of inversions between a sequence in intron 22 (int22h-1) of the F8 gene and one of two more distally located copies (int22h-2, int22h-3) described to be in the same orientation. Recently a novel finding from analysis of the DNA sequence of the human X chromosome (Ross et al, Nature 2005) has revealed that the two distal copies are in opposite orientations. Recombination should then produce deletion or duplication rather than inversion. A deletion consistent with this prediction has been reported in a family in which carrier females are affected by a high spontaneous-abortion rate in pregnancy. We report here the first case of duplication of exons 1 to 22 in a carrier female in a family where none haemophilic patient was available. The duplication was detected by MP-LC (Multiplex Liquid chromatography) and confirmed by MLPA. We believe this new rearrangement could be linked to the multiple copies of the intron 22 sequences and would confirm the hypothesis that recombination could produce duplication.

**P05.063****A comparison of quantitative real time PCR (Q-RT-PCR) and Multiple Ligation-Dependent Probe Amplification (MLPA) for molecular diagnosis of deletions in cases of severe haemophilia A**

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Haemophilia A (HA) is an X-linked bleeding disorder caused by mutations in coagulation factor VIII. The identification of HA carriers is an essential part of genetic counselling. Large rearrangements frequently occur within the F8 gene in severe haemophiliacs. These include the intron22 inversion (40-45%) the intron1 inversion (2-5%) and gross deletions encompassing one or more exons (5-10%). Although gross deletions are readily detectable in males, the identification of heterozygosity in possible carriers of these families constitutes a challenge. To identify a deleted allele over the background of the normal allele in these carriers, we previously set up a Q-RT-PCR method employing LightCycler technology. A comparison was performed with the recently described MLPA P178 FVIII probemix that contains probes for each of the 26 exons of the F8 gene. We studied patients with deletions in exon 13, in exons 23-25, in exon 15 and in exons 1-22. Carrier and non-carrier females from these families previously defined by quantitative or marker analysis were also tested. MLPA results in HA patients revealed the absence of the peak in the corresponding exon(s). There was a complete correlation with results in the carrier group (one copy of the corresponding exon(s) by Q-RT-PCR and 40- 55% of reduced relative peak area in MLPA) and also in the non carrier group (two copies and 85-100% of peak area). MLPA may be incorporated into routine molecular diagnosis of severe HA after screening of inversions. Supported by Fundaci Catalana d'Hemofilia, CIBERER, Real Fundaci Victoria Eugenia.

**P05.064****Investigation of human mitochondrial DNA in Iranian Hypertrophic Cardiomyopathy (HCM) patients**

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<sup>1</sup>NRCGEB, Tehran, Islamic Republic of Iran, <sup>2</sup>Russian Research Center of Medical Genetics Laboratory of DNA Research, Moscow, Russian Federation. 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 X 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.

**P05.065****Investigation of 69 common mutations in MYH7 gene in Iranian population with hypertrophic cardiomyopathy**

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Hypertrophic cardiomyopathy is characterized by hypertrophy of ventricles and intraventricular septum. Patients could develop serious complications including heart failure, arrhythmias and sudden death. Familial hypertrophic cardiomyopathy is a single gene disorder and has autosomal dominant inheritance. In this study we focused on exons 13-15 and 19-21 of **MYH7** gene and introns located between them, which contain hotspots for so called "malignant mutations" that increase sudden cardiac death risk. Methods: Fifty unrelated Iranian patients with hypertrophic cardiomyopathy were selected sequentially and informed written consent was obtained from them. Exons 13-15 and 19-21 of **MYH7** gene and their related introns were amplified by polymerase chain reaction. Then PCR products were sequenced. Results: Mutations were detected in fourteen (28%) of the patients. We didn't find any malignant mutation, but three mutations were found in targeted exons. One of them, A10419C (N444T) in exon 14, may be a novel mutation.

**P05.066****A novel mitochondrial DNA tRNA<sup>Leu</sup> (m.4322dupC) mutation associated with idiopathic dilated cardiomyopathy**

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Dilated cardiomyopathy (DCM) is a heart muscle disease characterized by cardiac dilatation and impaired contraction of the left ventricle (LV) or both ventricles. The age at disease onset is highly variable, ranging from early childhood to late adulthood. Only 50% of patients with DCM survive >5 years after diagnosis. Approximately 20% to 25% of cases seem to have a genetic component. DCM can be transmit-

ted as autosomal dominant and recessive, X-linked, or mitochondrial traits. Mitochondrial DNA (mtDNA) defects are found in an increasing number of cases of DCM. We identified a novel heteroplasmic mitochondrial DNA (m.4322dupC) mutation in tRNA<sup>le</sup> gene associated with isolated dilated cardiomyopathy (DCM) as maternal trait. Mutation screening techniques and automated DNA sequencing were performed to identify mtDNA mutations and to assess heteroplasmy in family's proband and healthy control subjects. All family members tested had heteroplasmic mtDNA (m.4322dupC) mutation. We also screened 350 normal controls for this mutation and found no evidence of heteroplasmy.

The m.4322dupC mutation was found in the skeletal tissue from the proband that exhibited slightly reduced deficiency of mitochondrial respiratory chain enzymes (complex III). The present study reports the novel m.4322dupC mutation in tRNA<sup>le</sup> gene, which is possibly associated to the disease, to isolated DCM. It was localized in a hot-spot region for mutations and is possibly pathogenic because of a cosegregation with the matrilineal transmission of DCM.

#### P05.067

#### **Analysis of the 12SrRNA and tRNAser(UCN) genes (mtDNA) in patients with nonsyndromic hearing loss from different Russia regions**

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Hearing loss is common congenital disorder and more than 50% of deafness has a genetic cause. The m.1555G>A (12SrRNA) was confirmed as the main cause of aminoglycoside induced deafness in different populations. Families with maternally inherited sensorineural hearing loss are also described in association with A7445G, T7511C, and 7472insC mutations in the tRNAser(UCN) gene. We report here the results of mutational screening for 12S rRNA and tRNAser(UCN) genes among Cx26- and Cx30-negative deaf individuals of different ethnicity from different regions of Russia. Previously, 301 unrelated patients from Volga-Ural region, 78 unrelated patients from the Republic Sakha (Yakutia, northeastern Siberia), and 119 deaf patients (75 unrelated families) from the Republic Altai (south Siberia) were analyzed for Cx26 and Cx30 mutations. Different variations at the 961 position in 12S rRNA gene have been found among deaf individuals from Volga-Ural region. Five patients of different ethnicity (Russian, Tatar, Latvian) with m.961insC, two Tatars with m.del961TinsC<sub>n</sub>, three Russian patients with m.961T>G (one from Volga-Ural region and two from Altai), and one Russian with m.961T>A, were detected. Also, the m.7444G>A (tRNAser(UCN)) was found in one Russian patient with NSHL. . Finally, m.7445G>C (tRNAser(UCN)) was found in two sibs of one Kazakh family (Altai region) in whom moderate sensorineural hearing loss was co-existed with goiter. Further studies are needed to confirm pathogenicity of some mtDNA variations associated with deafness in patients from some regions of Russia.

#### P05.068

#### **Prevalence of the GJB2 mutations in Iranian patients with deafness**

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The commonest form of non-syndromic recessive deafness is caused by mutation in GJB2, encoding gap junction beta 2 protein on chromosome location 13q11. It is known as DFNB1 responsible for half of autosomal recessive non-syndromic deafness. The most frequent mutation 35delG accounts for about 60-80% of mutations in white people of European.

In this study, we report the frequency of the GJB2 gene mutations in 31 unrelated Iranian families from 48 subjects affected by hereditary hearing loss (HHL).

Eight different mutations were detected in 12 families (38.7%) by using direct-sequencing technique in coding region of GJB2 gene. Cx26 related deafness mutations (35delG, R127H, V27I+E114G, Y155X, M163V and a novel 355-356 delGA) were identified in 9(29%) families

in heterozygous form, 3(9.67%) (35delG/35delG and R143W/ R143W) and 1 (3.2%) (R32H+35delG) were homozygous and compound heterozygous respectively.

Two polymorphisms V153I and (F154F +F146F) also were detected in four families and a polymorphism S86T was identified in all cases. In this population study, our data showed that the rate of GJB2 mutations is high in heterozygous form so other loci and genes related to deafness must be investigated. Moreover, the most frequent mutation was 35delG because 9 out of 18(50%) mutant alleles had this mutation. This is lower than that reported in western populations

#### P05.069

#### **Hereditary Congenital Hearing Loss: molecular analysis of Connexins 26, 30 and A1555G Mitochondrial Point mutation in Italian population**

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Mutations in GJB2, encoding the gap-junction protein Connexin26, are the most common cause of non-syndromic hearing loss (NSHL) and account for about 32% of cases in the Caucasian population. We analyzed 852 patients and identified mutations in 527/1662 chromosomes. We characterised 40 different mutations and 6 polymorphisms in 299 NSHL patients. More than 100 different mutations are described but one is particularly common, the 35delG, accounting for about 68% of all the Cx26 alleles we identified. The GJB6 gene deletion, del(GJB6-D13S1830), which can cause hearing loss in combination with GJB2 mutations in trans, has been found in 3 of our patients, while the del(GJB6-D13S1854) was not present in our patients' population. Our results show that GJB2/GJB6 genes account for less than 30% of NSHL in the screened cohort of patients and confirm that the 35delG mutation is the most frequent one. Moreover, 27 affected subjects were compound heterozygous for recessive GJB2 allele not including 35delG and 8 were carrying dominant mutations (T55N, P58A, D179N and R184Q), indicating that the complete sequence of the gene is needed for an appropriate molecular diagnosis. The analysis of the deafness-causing A1555G substitution in MTRNR1 mitochondrial gene was carried out in patients with one or without Cx26 recessive mutations. We found 21 affected subjects carrying the A1555G and the subsequently family analysis performed in each case has led to the pre-symptomatic identification of this mutation in relatives.

#### P05.070

#### **GJB2 analysis in Portuguese cochlear implant users**

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Hearing impairment affects approximately 1 in 1000 newborns and at least 60% of these cases have a genetic origin. Despite large genetic heterogeneity, mutations in a single gene - GJB2 - are the most frequent genetic cause of severe to profound pre-lingual recessive deafness in many populations. Therefore, GJB2 became the most important gene in the understanding of deafness. Hearing loss is a condition that interferes with the development of the child at a cognitive and language level. Therefore early diagnosis of deafness is important for earlier rehabilitation, namely through the use of cochlear implants. These devices replace the cochlea in a physiological context. Some studies suggest a correlation between the GJB2 genotype of the implanted individual and a phenotype that allows the success of rehabilitation due to cochlear implant

The aim of our study is to analyse the GJB2 gene in a sample of 100 Portuguese cochlear implant recipients. All individuals, presenting non-syndromic sensorineural severe to profound bilateral recessive deafness prior implantation, were implanted in the Centro Hospitalar de Coimbra. Screening of GJB2 gene was performed by PCR and sequencing in of the entire coding region. The results obtained may represent a valuable indicator when counselling candidates for cochlear implantation.

**P05.071****Characterization of the Dfna5 promoter region**

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DFNA5 mutations cause a non-syndromic, progressive, sensorineural hearing loss in humans. Four different mutations have been identified at the genomic level, all leading to exon 8 skipping at the mRNA level. As a consequence, it was hypothesised that DFNA5-related hearing loss is associated with a gain-of-function, and that only skipping of exon 8 leads to disease. With the objective to clarify the molecular basis of this gene's regulation, we have characterized the mouse *Dfna5* promoter region.

Initially, *in silico* analyses of the mouse *Dfna5* promoter region were performed followed by 5'-RACE experiments using mouse cochlear cDNA. The latter enabled us to identify the cochlear *Dfna5* transcription initiation site (TIS) *in vitro*. Subsequently, constructs were generated for transfection experiments in HEK293 cells. After confirmation of the core promoter region in a 400bp construct surrounding the suspected TIS, constructs of increasing length were generated to identify regulatory elements. Both an enhancer and a silencer element could be identified in the region upstream of the TIS. Next, transfection experiments performed with the organ of Corti cell line OC-k3 demonstrated that the suspected core promoter also drives expression in inner ear cells. Furthermore the enhancer and silencer elements act similarly in OC-k3 cells. Transfections using the 400bp construct in reverse orientation were performed as negative control. However, this construct also revealed promoter activity, suggesting the presence of an anti-sense regulatory element. Finally, transcription factor binding sites in the *Dfna5* regulatory region where identified using several computer modelling programs.

**P05.072****Mutational screening of GJB2 non-coding regions in Portuguese hearing loss patients**

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Many hearing loss mutations in the *GJB2* gene have been described in the last decade, being most of them located within the coding region. During this period, only a few mutational studies were performed on the non-coding regions of the gene. Such studies focused on the first exon, the donor splice site and, occasionally, the promoter region. None, to our knowledge, has ever included the whole 3' UTR. Two pathogenic mutations have so far been reported in *GJB2* donor splice site, and recently we have found a novel pathogenic mutation, -3438 C>T, occurring in the *GJB2* basal promoter. These mutations, or novel ones, in the *GJB2* non-coding regions may therefore be involved in other unelucidated cases of hearing loss.

In this study, we analysed by sequencing the *GJB2* promoter, exon 1, donor splice site and 3'UTR of about 100 unrelated Portuguese patients previously screened for *GJB2* coding region mutations.

An interesting finding was the identification of one homozygote for the -493del10 deletion upstream *GJB2* basal promoter, without any accompanying *GJB2* coding mutation. The significance of this mutation is yet unclear. However, in a previous study, over 6% of carriers, but no -493del10 homozygotes, were found in a control sample of 630 individuals, which might suggest this could be a pathogenic recessive mutation. Results regarding other non-coding variants are being assessed.

These data, and the previous reports on pathogenic non-coding *GJB2* mutations, justify routine screening of these regions in order to improve molecular diagnostic and genetic counseling of hearing loss patients.

**P05.073****Genetic etiology and spectrum of mutations in GJB2 and SLC26A4 genes**

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Heredity hearing impairment clinic was established at our institute in October 2003. 226 families have been investigated until October 2007. Genetic etiology of hearing impairment was found at 70,35 % of families (53,46% with autosomal recessive inheritance, 15,10% with autosomal dominant inheritance and 13,83% with genetic syndromes, chromosomal aberrations and another genetic diseases with deafness, at 17,61 % families the mode of inheritance could not be determined). The rest (29,65%) were families with idiopathic hearing loss.

The mutations in the *GJB2* gene (*Cx26*) were investigated at 321 patients by sequencing of entire coding region of *GJB2*. In patients carrying only one pathogenic mutation the IVS 1+1 G to A mutation in the non-coding region was further tested. We found 4 mutations not reported before (Ala149Thr, Ile140Ser, c.683+3 C to A, Gly130Val). At least one pathogenic mutation was found at 157 (48,91%) patients. Both pathogenic mutations were detected at 65 (20,25%) patients. No pathogenic *GJB2* mutation was detected at 134 (41,74%) patients and 30 (9,35%) patients are carriers of various polymorphisms or mutations not reported before. The mutation 35delG is by far the most common of all pathogenic mutations and with mutations Trp24Stop, 313del14 and -3170 G to A accounted in total for 95% of all causal mutated alleles in all patients.

Molecular genetic analysis of *SLC26A4* gene was introduced in Czech Republic in 2006. 23,08% patients with congenital deafness, goiter or enlarged vestibular aqueduct (EVA) have both pathogenic mutations of *SLC26A4* gene.

**P05.074****A Systems Biology Approach to Hearing: Combining Genomic, Proteomic and microRNA Characterization**

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Systems biology involves studying the interaction and interplay of many levels of biological information. We have combined transcriptomic and proteomic analyses of cochlear and vestibular sensory epithelia in order to identify networks of genes and proteins essential for the development and function of these inner ear organs. We further identified microRNAs (miRNAs) that are uniquely expressed in the auditory and vestibular sensory epithelia using bioinformatics tools and experimental approaches, including microarray profiling and *in situ* hybridization.

Expression profiling of vestibular and cochlear sensory epithelia using Affymetrix microarrays and proteomics analysis using the Q-TOF mass spectrometer with iTRAQ labeling has led to the identification of genes and protein networks which function differently in the cochlear and vestibular systems. A network analysis was applied to determine if a set of proteins of interest are physically connected, assuming that physical interaction between proteins points to some common function/pathway/complex. Two major sub-networks emerged from the integrated transcriptomic-proteomic clusters, indicating multiple interactions between proteins expressed in the cochlear and vestibular systems.

miRNAs are recognized as important regulators of gene expression at the post-transcriptional level and mutations in miRNAs can lead to disease. Combining our transcriptomic and proteomic data with miRNA identification in the inner ear has led us to make predictions regarding putative targets, which are being experimentally validated. For a number of miRNAs, morpholino experiments in zebrafish demonstrated abnormalities in inner ear development and/or structure, demonstrating the importance of these miRNAs in the inner ear. These miRNAs are candidates for causing deafness.

**P05.075****Three novel mutations in SLC40A1 gene causing hemochromatosis type 4**

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The Hemochromatosis type 4(OMIM : 606069, 2q32) is caused by mutations in the SLC40A1 gene which encodes the ferroportin, a 10 transmenbrane domains protein's which export iron from enterocytes and RE cells to blood transferrin. It is supposed to be regulated through interaction with the Hepcidin. Nowadays, 21 mutations and 7 polymorphisms of the SLC40A1 gene are described. It is the most frequent etiology for the "non HFE" hemochromatosis and it is inherited as an autosomal dominant trait. We found common mutations in SLC40A1 in 13 from 93 families referred to the laboratory for a "non HFE" hemochromatosis : G490D, G490S, G80S, V162Del and Q248H. The Study of three families which do not displayed a typical HH 4 iron overload (hyperferritinemia and low transferrin saturation), allowed us to discovered 3 new mutations : c. 212C >T (p.Ser71Phe) in exon 3, c.697C>A (p.ala232Asp) in exon 6 and c.797T>C (p.Met266Thr) in exon 7. All patients were heterozygous for the mutation and the autosomal dominant inheritance has been proven for p.Ser71Phe mutation since HH type 4 and mutation were present in mother and son. Interestingly, from those 3 mutations two are located in the loop between the TM domain 4 and 5 (p.ala232Asp and p.Met266Thr) where are located 11 from the 21 mutations already described giving more evidences for the role of this loops in the function or the regulation of this protein. This work highlight the frequency of HH type 4 and brings data to better understands the function of the protein.

**P05.076****Novel SLC40A1 mutations in Centre-South Mediterranean families with autosomal dominant iron overload.**

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Iron overload due to mutations in ferroportin is the most common form of "Non HFE hemochromatosis". Ferroportin disease or hemochromatosis type IV has a dominant inheritance and two main clinical phenotypes. Most cases show early increase in serum ferritin in the presence of a low-normal transferring saturation, iron loading predominantly in reticuloendothelial cells and, sometimes, low tolerance to the phlebotomy program. On the contrary, some cases are similar to the typical "classical hemochromatosis" characterized by early high transferring saturation and prevalent parenchimal iron overload.

Different kind of mutations in the iron exporter ferroportin (SLC40A1) result in hemochromatosis type IV. Loss-of-function mutations cause an impairment of iron export from reticuloendothelial cells with tissue iron accumulation but decreased availability of iron for circulating transferrin. Instead a phenotype overlapping with 'classical hemochromatosis' is the result of gain-of function mutations in SLC40A1.

We describe four novel missense mutations and a rare polymorphism (p.Arg561Gly) in ferroportin (SLC40A1) found in members of five different families of Centre-South Mediterranean showing autosomal dominant iron overload. Both loss-of-function (p.Ala69Thr; p.Asp181Asn) and gain-of-function (p.Arg296Gln; p.Tyr501Cys) mutations are recognizable in these families, so that a genotype-phenotype correlation is possible.

**P05.077****Detection of large duplications within the FVIII gene by MLPA**

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Haemophilia A is caused by a variety of different mutations in the FVIII gene: missense and nonsense mutations, small and large deletions and insertions, as well as large inversions. So far, only one duplication of a whole exon of the FVIII gene, exon 13, has been published by an Italian working group. Duplications comprising whole exons are dif-

ficult to detect by the usually applied methods for mutation screening in X-chromosomal linked disorders.

In about 80 haemophilia A patients (from a total of approx. 2000) we could not identify any mutation by long range PCR, DGGE and additional sequencing of all 26 exons and flanking intronic regions of the FVIII gene. These patients were investigated by multiplex ligation-dependent probe amplification (MLPA, MRC-Holland) for large duplications. We detected eight exon-spanning duplications of different length in nine haemophilia A patients. In two patients we found duplications of only one exon: exon 13 and 14, respectively. The other duplications affected more than one exon: exons 1-5, exons 5-25, exons 23-25, exons 2-25, exons 14-21 and exons 7-11, respectively. All duplications were confirmed by DHPLC analysis (denaturing high performance liquid chromatography).

In conclusion, we found large duplications in the FVIII gene to be causative mutations for haemophilia A in about 10% of all pre-screened patients in which we could not detect any mutation by the conventionally used methods.

MLPA is a convenient method for detection of large duplications and also deletions of whole exons particularly in X-chromosomal linked disorders as haemophilia A.

**P05.078****Frequency of factor VIII intron-1 inversion among hemophilia A patients from Republic of Macedonia and Republic of Bulgaria**

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Hemophilia A (HA) an X-linked bleeding disorder, is characterized by a wide allelic heterogeneity, as numerous mutations are spread out through the whole exonic, intronic and regulatory regions of the factor VIII gene. The most recurrent molecular defect is the inversion involving the intron-22, accounting for >40% of the patients with severe disease. Recently, Bagnall et al. (Blood, 2002) reported an inversion of intron-1 as a further recurrent mutation. The prevalence of this mutation is between 0.6 and 5%, according to reports from different groups.

The aim of this study was to determine the frequency of intron-1 inversion among 57 severe hemophilia A patients from Republic of Macedonia and 40 patients from Republic of Bulgaria. DNA samples from all 97 patients were analyzed for the presence of intron-22 inversion. Patients negative for the intron-22 inversion, were screened for the intron-1 inversion. Two PCR reactions flanking each *int1h* repeat (intronic and extragenic) were used.

We have identified the presence of intron-1 inversion in four out of 57 (7.0%) severe HA patients from Republic of Macedonia. None of these patients exhibited inhibitor development. Analysis of the factor VIII gene haplotypes demonstrated that the intron-1 breaking inversion has occurred independently in three (out of four) patients. No intron-1 inversion was detected among HA patients from Bulgaria.

Our data highlight the importance of analysis of the intron-1 inversion in the severe HA cases from Republic of Macedonia. This will benefit both genetic counseling and the study of the relationship between genotype and inhibitor development.

**P05.079****Molecular analysis of a novel deletion at Exon 4 of factor IX gene in a hemophilia B patient**

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Hemophilia B (HB) 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 kb of genomic DNA.

Molecular analysis of F9 gene in a severe Iranian HB patient and carrier testing for the family referred from Kashan hemophilia center.

After obtaining informed consent, genomic DNA was extracted from the peripheral blood of the patient and his mother and sister by standard methods. PCR amplification and single strand conformation poly-

morphism (SSCP) techniques were performed for scanning of the all functional-important regions of the F9 gene. An abnormal SSCP profile was identified in exon 4 of the gene for this patient. Then, direct sequencing was done by chain termination method.

The deletion of 19 nucleotides ( 10503-10521 del ) was detected in this patient in exon 4

which is not reported in hemophilia B mutation database, previously. The patient's mother and his sister were heterozygous for this deletion.

A novel deletion of 19 nucleotides (10503-10521 del ) in exon 4 leading to frame shift in the epidermal growth factor1( EGF1) domain. Exon 4 encoded a first epidermal growth factor-like domain, which shows homology to epidermal growth factor (EGF) and, in addition, contains conserved carboxylate residues including a  $\beta$ -hydroxyaspartate at amino acid 64. This domain binds an additional  $\text{Ca}^{2+}$  with high affinity. In conclusion, this deletion causes a severe form of disease as observed in this patient.

#### P05.080

#### Complete Maternal Isodisomy Causing Reduction to Homozygosity for a Novel *LAMB3* Mutation in Herlitz Junctional Epidermolysis Bullosa

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Herlitz junctional epidermolysis bullosa (HJEB) is a very rare lethal genodermatosis characterized by blister formation with tissue separation within the lamina lucida of the basement membrane zone. It is genetically heterogeneous, being caused by null mutations in the *LAMA3* (18q11.2), *LAMB3* (1q32), or *LAMC2* (1q25-q31) genes encoding for the alpha3, beta3 and gamma2 subunits of laminin 332. Although it is usually inherited in an autosomal recessive fashion, four cases have been described in which the disease arose from reduction to homozygosity for a mutant allele caused by uniparental disomy (UPD). Here, we report on a baby presenting with multiple blister formation at birth and who died due to acute respiratory insufficiency secondary to upper airway obstruction at 1 year of age. The diagnosis of HJEB was first confirmed by immunoepitope mapping of a skin biopsy. Molecular analysis identified a novel frameshift mutation at the homozygous state in *LAMB3*. Parental genotyping established the mother as a healthy carrier, while in the father the mutation was absent. Haplotype analysis of 8 intragenic and 15 extragenic polymorphisms, spanning the entire chromosome 1, demonstrated that the proband was homozygous for a single maternal haplotype. The present case represents the fifth example of UPD in HJEB. Based on literature data and our experience in genotyping 20 HJEB patients from several European countries, reduction to homozygosity due to UPD is not exceptional in this condition and should be considered in sporadic cases in order to properly counsel the couple.

#### P05.081

#### Genetic causes of hearing loss in the Iranian population a 10 year study

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Approximately one in 1000 newborns has severe-to-profound deafness, which has a genetic basis in more than half of this group. In ~70% of congenitally deaf newborns the loss is nonsyndromic.

The purpose of this study has been to determine the cause of syndromic and non-syndromic hearing loss in the Iranian population.

Over a 10 year period, 2434 families segregating deafness have been referred to Genetics Research Center in Tehran. In each of these families, informed consent was obtained. Every family was screened for mutations in *GJB2* and *GJB6*. Following this screen, in 300 families with three and more affected individuals with presumed ARNSHL, we

screened total of 22 loci by STR markers. In families in which autozygosity by descent was not identified at any of these loci, a genome-wide screen was completed.

Our data show that 15.3% of the Iranian population with ARNSHL segregate mutations in *GJB2*. The most prevalent mutation in this gene was 35delG, although more than 20 mutations have been identified, five of which are unique to the Iranian population. We also identified novel *GJB2* mutation in an endogenous population segregating ADNSHL in village north of Iran.

The second and third most prevalent causes of ARNSHL were mutations in *SLC26A4* and *TECTA*. We have also found mutations in *PJVK* and *TMC1* in one family each.

We have identified a number of families segregating syndromic hearing loss. Totally, we have been able to determine the genetic cause of hearing loss in over 30% of families referred to our center.

#### P05.082

#### The investigation of *NPHS1* gene in children with Hereditary Proteinuria Syndrome

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Hereditary Proteinuria Syndromes (HPS) is a group of inherited diseases in which proteinuria is the primary clinical manifestation. The genetic cause of these diseases is mutations in genes providing the structure and function of filtration barrier.

DNA from 63 children with idiopathic nephrotic syndrome in age are ranged from 1 to 17 years, consisting of 33 boys and 30 girls, have been analyzed. The disease manifestation had been observed at the age ranged from 1 month to 16 years. The control group has consisted from 50 healthy persons. The mutations in *NPHS1* gene were investigated by using SSCP-analysis and consequent sequencing.

No mutations in coding and regulation regions of this gene were found.

At researched patients has been revealed five single nucleotide substitution (table 1), one of which c.605-20 A>C has not been described earlier. For specification of influence of this substitution by occurrence of Proteinuria Syndrome, we investigated frequency of occurrence in control sample. Authentic distinctions have not been revealed.

We have not found significant distinctions allele frequency of four others polymorphisms *NPHS1* gene between our patients and control group. We think that contribution of these polymorphisms to disease is unimportant.

Minor allele frequency of polymorphisms *NPYS1* gene in our patients and control group (%)

exon	polymorphism	HPS group	control group	Fisher exact test (P)
Ex3(c.349G>A)	c.349A	25	26	0.87
Ex10(c.1175C>T)	c.1175T	2	4	0.45
Ex10(c.1223G>A)	c.1223A	4	3	0.72

Minor allele frequency of polymorphisms *NPYS1* gene in our patients and control group (%)

exon	polymorphism	HPS group	control group (our data)	Fisher exact test (P)
Ex7(c.791C>G)	c.791G	1	1	1
Ex6(c.605-20A>C)	c.605-20C	1	0	0.5

#### P05.083

#### Analysis of *SPASTIN* in a Spanish series shows both recurrent and novel mutations

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Compostela, Spain.

Hereditary spastic paraparesis (HSP) is a progressive gait disorder with spasticity of the limbs. HSP shows both clinical and genetic heterogeneity and can be autosomal dominant (AD-HSP), autosomal recessive or X-linked recessive. The most prevalent form of AD-HSP involves the *SPG4* locus encoding spastin, a member of the AAA family of ATPases. The frequency of *SPG4* mutations in our population is unknown. We studied 56 patients (43 families, 33 of them Galician, 10 from other regions of Spain), most with uncomplicated forms of HSP. The 17 exons of *SPG4* and exon-intron boundaries were sequenced. Novel mutations and sequence variations of unknown significance were checked in 186 Galician controls. We identified six mutations, three of them novel. The same truncating mutation was present in four apparently unrelated pedigrees. We found four sequence variations of unknown significance, one synonymous polymorphism and six other sequence alterations not found in the literature or databases but unlikely to be pathogenic (either present in controls or not co-segregating with the phenotype when several affected family members were available). In summary, 9/43 families (21%) were positive for *SPG4* mutations and another four families (9%) harbour sequence variations of unknown significance. If we consider only the cases with definitive autosomal-dominant inheritance, the frequency of *SPG4* mutations rises up to 60% in our series, confirming that *SPG4* causes most of the AD-HSP also in our community. In the cases with no mutations the cause may be in other genes or mutations not detectable by sequencing of the coding region.

#### P05.084

#### Mutational analysis of the *ACVR1* gene in Italian patients with Fibrodysplasia Ossificans Progressiva (FOP)

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<sup>1</sup>G. Gaslini Institute - Laboratory of Molecular Genetics, Genova, Italy, <sup>2</sup>National Cancer Research Institute, Genova, Italy, <sup>3</sup>G. Gaslini Institute - Laboratory on Pathophysiology of Uremia, Genova, Italy, <sup>4</sup>University of Pennsylvania School of Medicine, Philadelphia, PA, United States, <sup>5</sup>G. Gaslini Institute - second Unit of Pediatrics, Genova, Italy, <sup>6</sup>University of Genova and CEBR, Genova, Italy. Fibrodysplasia Ossificans Progressiva (FOP, MIM 135100) is a rare genetic disorder characterized by the presence of a congenital great toe malformation and progressive heterotopic ossification that transforms skeletal muscles to bone following a well-defined anatomic pattern of progression. Heterotopic ossification begins in childhood, either spontaneously or upon induction of stimuli such as trauma, and progresses episodically throughout adulthood. FOP is usually sporadic, however, some familial cases with an autosomal dominant pattern of inheritance with variable expression have been described. Linkage analysis in these families led to identification of *ACVR1/ALK2* as the gene responsible for FOP. All familial and sporadic cases with a classic FOP phenotype that have been analysed are heterozygous for the identical mutation, c.617G>A leading to R206H substitution. The only reported exception is a recently described de novo mutation, G356R, associated with a slowly progressing form of FOP. The *ACVR1* gene encodes the activin A type I receptor, a serine/threonine kinase receptor for bone morphogenetic proteins (BMPs) belonging to the TGF-beta receptor family. In this study, we report *ACVR1* mutational analysis in a group of 17 Italian FOP patients. We confirmed the presence of the recurrent R206H substitution in 14 patients and we identified a novel mutation (R258S) in the *ACVR1* kinase domain in one patient. We used bioinformatic tools to predict functional effects on the protein caused by the identified mutations and constructed a 3D molecular model of the R258S mutant in order to gain a better understanding of the possible effects of this newly described mutation.

#### P05.085

#### Hereditary hyperferritinemia and cataract syndrome - genetic study of a Portuguese family from the Azores

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Hereditary hyperferritinemia cataract syndrome (HHCS) is an autosomal dominant disorder characterised by the presence of cataracts with

markedly elevated serum ferritin levels without iron overload. Ferritin is the major protein involved in iron storage and is composed of H and L subunits. The translation of these subunits depends on the iron status of the cell and is regulated by the interaction between the iron responsive element (IRE) present in the mRNAs 5' noncoding region and cytoplasmic iron regulatory proteins. HHCS is caused by mutations in the IRE of the ferritin light chain (FTL) gene.

In this study we have investigated the genetics of a three generation Azorean kindred with typical HHCS in 3 individuals (2F;1M). The proband was identified, after detection of hyperferritinemia, through the review of clinical findings and biochemical tests. Hereditary haemochromatosis frequent mutations in the *HFE*, *TFR2* and *FPN1* gene were screened. All participants gave informed consent before being included in the study.

DNA was extracted from whole blood. Promoter and coding regions of the human *FTL* gene were subjected to PCR amplification and sequenced bidirectionally. Screening of *HFE* mutations was performed by PCR-SSO.

The affected individuals are heterozygous for *HFE* mutation, H63D. Sequencing of *FTL* gene in the affected individuals identified a heterozygous mutation in position 47 relative to transcription initiation site in the descending part of the IRE (47G>A). None of the unaffected individuals from the family had the referred mutation.

#### P05.086

#### Identification of eight novel mutations in German patients with Hereditary Hemorrhagic Telangiectasia (HHT) and detection of a significant association between mutations in the *ACVRL1* gene and liver involvement

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Background: Hereditary hemorrhagic telangiectasia (HHT), or Osler-Weber-Rendu disease, is an autosomal dominant inherited disorder of the vascular tissue. This genetically heterogeneous multi-systemic dysplasia shows a wide variation in its phenotypic expression. Up to 78% of the HHT patients show hepatic arteriovenous malformations (HAVM), but the molecular basis of liver involvement is still unknown. In approximately 75% of the patients, mutations can be identified by sequencing of the two known HHT genes, *ACVRL1* (*ALK1*) and *ENG*. Genotype-phenotype correlations are not yet fully defined, but previously, we and others showed that hepatic involvement is associated with *ACVRL1* mutations, but rarely caused by mutations in the *ENG* gene.

Patients and methods: In a new cohort of 18 adult HHT patients we performed sequencing analysis of the *ACVRL1* and *ENG* gene.

Results: Eight novel and 8 already described mutations (10 missense mutations, 2 small in-frame deletions, 3 premature stop mutations and 1 small frameshift deletion) were identified. Analysis of our entire data revealed statistically significant differences in the distribution of *ACVRL1* and *ENG* mutations among HHT patients with and without HAVM ( $p=0.0016$ ).

Conclusion: Our data support the growing evidence for a significant correlation between mutations in the *ACVRL1* gene and liver involvement in HHT, and suggest that molecular genetic testing in HHT patients is important for prognosis with respect to liver disease.

**P05.087****The GNE protein binds to alpha actinin 1**

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Hereditary inclusion body myopathy (HIBM) is a rare neuromuscular disorder caused by mutations in *GNE*, the key enzyme in the biosynthetic pathway of sialic acid. While the mechanism leading from *GNE* mutations to the IBM phenotype is not yet understood, we searched for proteins potentially interacting with *GNE*, which could give some insights about novel putative biological functions of *GNE* in muscle. We used a Surface Plasmon Resonance (SPR)-Biosensor based assay to search for potential *GNE* interactors in anion exchanged fractions of human skeletal muscle primary culture cell lysate. Analysis of the positive fractions by *in vitro* binding assay revealed  $\alpha$ -actinin 1 as a potential interactor of *GNE*. The direct interaction of the two proteins was assessed *in vitro* by SPR-Biosensor based kinetics analysis and in a cellular environment by a co-immunoprecipitation assay and confocal co-localization in 293T cells. The interaction of *GNE* with  $\alpha$ -actinin 1 might point to its involvement in  $\alpha$ -actinin mediated processes, including cytoskeleton organization and signaling pathways. In addition these studies illustrate for the first time the expression of the non-muscle form of  $\alpha$ -actinin,  $\alpha$ -actinin 1, in mature skeletal muscle tissue, opening novel avenues for its specific function in the sarcomere.

**P05.088*****NTRK3*, a gene involved in the enteric nervous system development, is related to Hirschsprung disease**

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Hirschsprung disease (HSCR) is a developmental disorder characterized by the absence of ganglion cells in the myenteric and submucosal plexuses along a variable portion of the distal intestine, due to a defect of neural crest neuroblasts migration process. Manifestation of the disease has been linked to the dysfunction of 2 principal signalling pathways involved in the enteric nervous system (ENS) formation: the RET-GDNF and the EDN3-EDNRB receptor systems. Because of its etiopathogenesis it results tempting to speculate that additional signalling pathways implicated in intestinal neurodevelopment could also be involved in HSCR. In this way, the NTF3/TrkC signalling pathway had been shown to play an essential role in the development of the ENS, together with the evidences showed by murine models lacking or over-expressing NTF-3, and the differential localization of the receptor in ganglionic *versus* aganglionic region of HSCR intestine suggest a potential role for those genes in the pathogenesis of HSCR. We have sought to evaluate the candidature of the *NTRK3* gene, encoding the TrkC receptor, as a susceptibility gene for Hirschsprung disease. Using direct sequencing analysis and dHPLC technology we have screened the *NTRK3* coding region and the intron/exon boundaries in 143 Spanish HSCR patients. A total of 4 previously described polymorphisms and 12 novel sequence variants were detected. Of note, we have detected a novel aminoacid substitution in the protein sequence in a multiplex HSCR family. *In silico* studies point that structural alterations might be introduced in the mutated protein, suggesting a pathogenic role for Hirschsprung disease.

**P05.089****Nrf2-related oxidative stress response and impaired dopamine biosynthesis in a PC12 cell model of Huntington's disease**

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Huntington's disease (HD) is a devastating disease for which currently no therapy is available. It is a progressive autosomal dominant neuro-

degenerative disorder that is caused by a CAG repeat expansion in the HD gene, resulting in an expansion of polyglutamines at the N-terminal end of the encoded protein, designated huntingtin, and the accumulation of cytoplasmic and nuclear aggregates. Not only is there a loss of normal huntingtin function, upon expansion of the CAG repeat there is also a gain of toxic function of the huntingtin protein and this affects a wide range of cellular processes. To identify groups of genes that could play a role in the pathology of Huntington's disease, we studied mRNA changes in an inducible PC12 model of Huntington's disease before and after aggregates became visible. This is the first study to show the involvement Nrf2-responsive genes in the oxidative stress response in HD. Oxidative stress related transcripts were altered in expression suggesting a protective response in cells expressing mutant huntingtin at an early stage of cellular pathology. Furthermore, there was a down-regulation of catecholamine biosynthesis resulting in lower dopamine levels in culture. Our results further demonstrate an early impairment of transcription, the cytoskeleton, ion channels and receptors. Given the pathogenic impact of oxidative stress and neuroinflammation, the Nrf2-ARE signaling pathway is an attractive therapeutic target for neurodegenerative diseases.

**P05.090*****MyBPC3 (Myosin binding protein C) associated hypertrophic cardiomyopathy in young Maine Coon cats appears to be a recessive disease caused by the synthesis of mutated protein***

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Human hypertrophic cardiomyopathy (HCM) is a primary disorder of the myocardium associated with sudden death, stroke and heart failure. It is caused by mutations in genes coding for sarcomeric proteins. HCM in the Maine Coon cat (MCO) is a good spontaneous model of human HCM. Variants in the *MyBPC3* gene, coding for myosin binding protein-C (MYBP-C), are associated with human HCM. The mutation A31P in *MyBPC3* is associated with familial HCM in MCO and we screened a large cohort of unrelated MCO cats to establish the association between this mutation and feline HCM.

Two-hundred-eighty-seven MCO cats (mean age of 2.2 years, 23 with HCM) were genotyped for the A31P. Mutation screening was performed by DNA sequencing. Heart proteins were extracted from two MCO cats, separated by SDS-PAGE, the MYBP-C band was trypsin digested and sequence variants identified by mass-spectrometry (MS).

A31P had a minor allele frequency of 0.20. The odds ratio for having HCM was 10.7 (95%-cfi: 3.3 - 34.4) in homozygous cats. The presence of MYBP-C with the A31P variant in heart tissue was verified by MS in a homozygous MCO cat.

*MyBPC3* associated HCM in MCO express itself as a recessive disease in young cats, as a single A31P allele does not confer an increased risk of HCM. However, the potential for late-onset of disease may cause the significance of the genetic variant to be underestimated. The presence of the A31P variant MYBP-C in hearts suggests that the disease is caused by the presence of a "poisonous polypeptide".

**P05.091****Myocyte function and gene defects in arrhythmogenic right ventricular dysplasia: clinical phenotypes and open problems for clinical genetics**

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Background. Desmosome proteins' defects are associated with right ventricular (fibro)-fatty replacement of myocardial tissue (Arrhythmic Right Ventricular Cardiomyopathy/Dysplasia, ARVC/D, MIM #609040 and #107970). The most investigated genes are: JUP (Junctional Plakoglobin, 17q21), PKP2 (Plakophilin-2, 12p11), DSC2 (Desmocollin 2, 18q12.1), and DSG2 (Desmoglein 2, 18q12.1-q12.2), DSP (Desmoplakin, 6p24). We screened the five genes in a consecutive series of 65 patients with ARVC/D fulfilling (n=3) and non-fulfilling (n=62) McKenna et al. criteria and 55 DCM patients with arrhythmias, two with suspected myocarditis, and one with a wrong diagnosis of

## amyloidosis.

Methods. The ARVC/D was diagnosed on McKenna et al. criteria (1994), the DCM according to the WHO criteria. The coding and flanking regions of the five genes were analysed by direct automated sequencing of the heteroduplex amplicons.

Results. We identified 38 mutations in 110 index patients (24 of the 65 ARVC/D patients and 14 of the 55 DCM patients), with 4 patients carrying double/compound heterozygous mutations and 2 carrying a triple heterozygosity; the segregation of the gene defects with the phenotype was confirmed in the 3 probands who fulfilled the criteria of ARVC/D: two carried a missense and frameshift mutations and one a frameshift mutation.

Conclusions. The recurrence of double/triple mutants raises serious problems in molecular diagnosis and family study, especially when the family is small and segregation cannot be assessed, in particular the prediction of the development of the disease in young mutation carriers. Due to the current uncertainties, healthy mutation carriers should undergo regular clinical monitoring.

**P05.092****Development of a method for the genetic screening of 537 mutations associated with hypertrophic cardiomyopathy and analysis of a large cohort of patients**

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More than 500 different mutations have been associated with the development of hypertrophic cardiomyopathy (HCM). However, genetic diagnosis has not been till now a useful tool for clinicians because of its limited availability and high cost.

Objectives: the development of a genotyping platform to identify all the mutations that have been associated with HCM, to perform the screening of a large cohort of patients with HCM with this platform, and to evaluate the clinical factors associated with the presence of mutations.

Methods: We analyzed by Massarray SNPtyping 537 mutations in 15 genes: MYH7 (224), MYBPC3 (169), TNNT2 (37) TPM1 (11), ACTC (7), MYH6 (5), MYL2 (11), MYL3 (5), MYLK2 (1), MYO6 (1), PRKAG2 (9), TCAP (2), TNNC1 (3), TNNI3 (33) and TTN (9) in 773 consecutive index patients with HCM.

Results: We identified 74 different mutations (MYBPC3 34, MYH7 24, TNNT2 5, TNNI3 5, TPM1 2, TNNC1 1, MYH6 1, ACTC 1, MYLK2 1) in 163 different patients (21%) (MYBPC3 in 98 pts, MYH7 43, TNNI3 11, TNNT2 10, ACTC 7, MYH6 6, TPM1 2, MYLK2 2, TNNC1 1) (17 patients had 2 mutations and 1 was homozygous). Mutations were found in 100/392 pts from center C (26%), 37/147 from center M (25%) and 26/234 from center A (16%).

Conclusions: Genetic screening of known mutations in HCM provides the identification of mutations in 20 to 40% of our index patients. The likelihood of a positive diagnosis is higher in patients with familial disease and in patients with sudden death risk factors.

**P05.093****Unusual presentation of Kelley-Seegmiller syndrome**

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Kelley-Seegmiller syndrome means partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (HPRT), which is an X-linked genetic defect of purine metabolism. Characteristic features are hyperuricemia, nephrolithiasis and gout, resulting from uric acid overproduction. Female carriers have somatic cell mosaicism of HPRT activity, and are healthy with normal enzyme activity in erythrocytes. Only few females suffering from gout with this disorder were described.

We report a 50 year-old woman, who did not experience neither gout,

nephrolithiasis nor hyperuricemia. She was never on allopurinol. Uric acid was quantified by specific enzymic method and erythrocytes enzyme with plasma purine metabolites were measured by HPLC methods. Detailed purine biochemical investigations revealed in repeat serum uric acid concentrations within normal limits ( $314 \pm 12 \mu\text{mol/l}$ ); increased plasma levels of hypoxanthine:  $19.8 \mu\text{mol/l}$  (control values  $2.5 \pm 1.0 \mu\text{mol/l}$ ) and xanthine:  $7.5 \mu\text{mol/l}$  (control values  $2.4 \pm 0.7 \mu\text{mol/l}$ ); HPRT activity in erythrocyte lysate was surprisingly very low:  $8.6 \text{ nmol/h/mg Hb}$  (control values  $:113 \pm 11 \text{ nmol/h/mg Hb}$ ). Mutation analysis using direct sequencing revealed heterozygous form of previously described mutation in the 3<sup>rd</sup> exon of HPRT gene, c.215A>G (Y72C). Subsequent analysis showed skewed X-inactivation ratio in favour of mutant allele (> 25:75), which could explain enzyme defect. Although enzyme deficiency with urate overproduction (presenting as high plasma oxypurines) is evident, the reason for normal serum urate concentrations remains uncertain. Such results have not been reported in a female with HPRT deficiency. In conclusion our finding shows the need of detailed purine investigation in asymptomatic female members of family with partial HPRT deficiency.

**P05.094****Heterochromatic genes undergo epigenetic changes and escape from silencing in ICF syndrome**

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ICF (Immunodeficiency, Centromeric Instability, and Facial Anomalies), is a rare autosomal-recessive disorder. Most of the ICF patients were born from consanguineous marriages and about 60% have mutations in the DNMT3B, a *de novo* DNA methyltransferase. ICF syndrome is characterized by a marked immunodeficiency (patients tend to have low levels of immunoglobulins); facial anomalies are a heterogeneous trait and centromeric instability is the most typical feature of the disease. The juxtapacentromeric heterochromatin of chromosome 1, 9, and 16 is markedly undercondensed and is involved in chromosome rearrangements and multiradiate associations. The instability correlates with a severe hypomethylation of the classical satellites 2 and 3, which are the major components of constitutive heterochromatin. It is unknown how loss of DNA methylation in non-coding sequences accounts for the multiple symptoms associated with the ICF syndrome. Having observed that in tumor cells and cancer cell lines heterochromatic genes become hypomethylated and escape from silencing, we asked whether the same process is found in ICF syndrome. In this work we showed that heterochromatic genes are strongly hypomethylated and some of them escape from silencing in ICF cells relative to controls. Having observed that some heterochromatic genes remain silent, in spite of loss of methylation, we concluded that hypomethylation is necessary but not sufficient to have transcription in heterochromatic regions. ChIP experiments will show whether the activation of heterochromatic genes is regulated by histone modifications. Aberrant transcription of heterochromatic genes may contribute to some of the symptoms that are associated with ICF syndrome.

**P05.095****Clinical and Molecular study of SCN1A-related Epilepsy in Iranian families**

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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. SCN1A-related seizure disorders are inherited in an autosomal dominant manner. Most SCN1A-related Seizures are the result of a *de novo* heterozygous mutation. The proportion of cases caused by *de novo* mutations varies by phenotype. The percentage of probands with an SCN1A-related seizure disorder and an affected parent decreases as the severity of the phenotype in the proband increases. Material and methods: Diagnostic classification of patients followed on Classification and Terminology of the International League Against Epilepsy

(1989). Family History, Electroencephalography (EEG) recordings and CT Scan were obtained from most patients. The study was approved by the Iranian institutional ethics committees. DNA was obtained from 50 unrelated families with idiopathic generalized epilepsy as index families. The 26 exons of SCNIA were screened for deletions and duplications by MLPA. In order to find 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. We have identified some new intronic variants in SCNIA and new mutations too, in patients with IGE subtypes.

Allele and genotype frequencies in the patients and in the control groups were compared statistically.

#### P05.096

#### Molecular genetic analysis of SCN4B gene in Russian patients with idiopathic ventricular arrhythmias

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Cardiac channelopathies is a new class of diseases characterized by high risk of life-threatening arrhythmias and sudden cardiac death with apparently normal heart. Mutations in more than 10 different genes can cause these diseases. For different disorders within this group approximately 25-60% patients remain genotype negative. Recently, a new gene SCN4B encoding β4-subunit of Na<sub>v</sub>1.5 was shown in Long QT Syndrome (LQTS) family. The aim of this work is to screen mutation in SCN4B gene in patients with idiopathic ventricular arrhythmias and to estimate the prevalence of genetic alterations in this gene in Russian patients. The DNA samples of 48 unrelated patients (mutations in KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 had been excluded) with ventricular arrhythmias had been analyzed. Fourteen patients had LQTS, 14 patients had Brugada Syndrome (BrS) and 20 patients had Idiopathic Ventricular Tachycardia (IVT). Molecular genetic testing was performed by PCR-SSCP analysis with following direct sequencing of abnormal conformers. We did not find any disease-causing mutation in SCN4B gene. We suppose that SCN4B-associated arrhythmias are extremely rare in Russian patients with LQTS, BrS and IVT. Polymorphism c. C174T (p. C58C) in exon 2 was found within group of patients with BrS. The prevalence of this SNP among the patients with BrS and control group (100 unrelated unaffected individuals) was about 7% without significant differences. Thus, it's unlikely that c. C174C can significantly modify Na<sup>+</sup>-channel function. By now, routine analysis of SCN4B gene for patients without mutations in KCNQ1, KCNH2, SCN5A, KCNE1 and KCNE2 seems to be inexpedient.

#### P05.097

#### CS expression in infertile men correlates with the efficiency of the spermatogenic process

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Germ cells use mitochondrial Krebs cycle (KC)/electron transport system and glycolysis for the maintenance of their ATP concentration, but each stage of spermatogenesis rely on one or other pathway for ATP production. Interestingly, spermatocytes and spermatids RNA and protein synthesis is highly dependent on mitochondria as the major ATP supplier.

Citrate synthase (CS) is the first enzyme in the KC. We have assessed the role of KC in the progression of spermatogenesis by the CS gene expression analysis in testicular biopsies of 39 non-obstructive and 20 obstructive infertile men. Samples were classified into four groups, from 1 (total absence of germ cells) to 4 (conserved spermatogenesis), on the basis of the Johnsen score count.

The current method has been the real time RT-PCR [LightCycler™ Instrument (Roche) and SYBR Green I fluorescence dye] and the relative quantification strategy, using cyclophilin-A as an endogenous control gene. The Mann-Whitney U test was used to analyse gene expression differences between groups.

CS transcript levels positively correlated with all germ cell stages number but a negative correlation was found with Sertoli cells supporting

the greater contribution of germ cells to CS transcription. Transcript levels of CS were significantly decreased in spermatogenic failure-affected-samples ( $p=0.01$ ), suggesting to play a role in the efficiency of spermatogenesis. Additionally, its significant correlation with the elongated spermatid number may indicate that CS has an active transcription in the latest stages of spermatogenesis with potential implications in the metabolic activity of sperm.

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#### P05.098

#### Differential susceptibility of imprinting centers to epimutations under DNA demethylation influence

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Genomic imprinting is an epigenetic phenomenon, which is involved in regulation of embryonic development, placental function and neurobehavioural processes. Changes in differential methylation of imprinting control (IC) regions are one of the possible mechanisms leading to aberrant gene inactivation or loss of imprinting associated with some hereditary diseases and cancer. However, incidence of epimutations in different imprinted loci as well as underlined mechanisms of its arising remains elusive. Previously we have reported about differential susceptibility to epimutations of three IC's in spontaneous abortions and described the loss of methylation of KCNQ1OT1 in 9.5% of embryos. The aim of the present research was analysis of methylation stability of previously investigated IC's (SNURF-SNRPN, H19/IGF2, KCNQ1OT1) under influence of DNA demethylating agent such as 5-aza-2-deoxycytidine *in vitro*. Fetal fibroblasts cell cultures from 17 induced abortions (9.6±0.2 weeks of pregnancy) with normal karyotype were treated by 5-aza-2dC with 5 µg/ml for 72 h. Methylation analysis was performed by methyl-specific or methylation-sensitive PCR. A normal differential methylation of SNURF-SNRPN and H19/IGF2 was observed in all cell cultures after 5-aza-2dC treatment. As to KCNQ1OT1, all cell cultures have revealed a loss of methylation in maternal allele. Loss of imprinting in KCNQ1OT1 was observed in our previous research of spontaneous abortions as well as in most children born after IVF procedures with Beckwith-Wiedemann syndrome reported in literature. Our results indicate a different susceptibility of IC's to epimutations, providing evidence for KCNQ1OT1 as a «hot spot» of aberrant epigenetic modifications in the human genome.

#### P05.099

#### Infantile neuroaxonal dystrophy: challenges for identification and new advances in prenatal diagnosis

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Infantile neuroaxonal dystrophy (INAD; OMIM #256600) is a rare disease belonging to the group of neurodegenerative disorders together with NBIA (neurodegeneration with brain iron accumulation, PANK2 gene, OMIM #234200), KARAK syndrome and Schindler disease (OMIM #609241). These diseases share the pathologic feature of axonal degeneration with distended axons throughout the central nervous system. In previous work there has been some controversy regarding whether INAD is a separate entity or is part of a spectrum of diseases with panthothenate kinase-associated neurodegeneration (PANK). Based on molecular studies of the PANK2 gene it became clear that INAD and PANK are genetically heterogeneous disorders. Recently, a gene PLA2G6, encoding a phospholipase A2, has been recognized as causing INAD. However, there is still some overlap in phenotypes resulting from deficiencies in the PLA2G6 and PANK2 genes. We present here the clinical features, diagnosis and molecular results of four patients from three unrelated families in whom a definitive diagnosis could be made by molecular analysis.

In all four patients clinical data and biochemical studies were in agreement with a diagnosis of neuroaxonal dystrophy. Molecular studies of the PLA2G6 were performed to confirm this diagnosis. The two male siblings from family 1 were homozygous for a p.Val371Met substitution; there was no apparent consanguinity in this family. A healthy carrier female was born after prenatal diagnosis through chorionic villus

sampling. The 2 other patients were from consanguineous parents, and were homozygous for a previously described p.Leu481Gln mutation and a frameshift mutation resulting from a 2bp duplication, respectively.

#### P05.100

#### A new syndrome with infantile-onset spasticity, mental retardation and abnormalities of white matter and cerebellum in a large consanguineous family.

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We describe in a sibship of 11 children from consanguineous parents 2 apparently distinct disorders. Seven sibs suffer from autosomal recessive arterial tortuosity syndrome (ATS) caused by a homozygote GLUT10 mutation, as reported before (Coucke, Nat Genet, 2006). We classified the second disorder and report linkage studies to identify a distinct recessive locus. Five sibs present with a neurodevelopmental disorder, consisting of neonatal hypotonia, severe psychomotor retardation, no independent deambulation, progression to tetraplegia, excessive drooling, strabismus, no speech development, no signs of denervation, no seizures and prolonged survival. The oldest patient is 25 years old. Four of them have also ATS, but the fifth has no sign of arterial tortuosity and is heterozygote for the familial GLUT10 mutation. Brain MRI of this patient shows asymmetric widespread loss of periventricular white matter with normal myelination and cerebellar atrophy. MRI of the other neurologically affected sibs is similar, with the addition of arterial tortuosity. Brain autopsy of one of the five sibs who died of aspiration shows signs of neuro-axonal dystrophy. DTI and fiber track data analysis from MRI suggest a combined neuron and myelin damage. We undertook linkage analysis for a second recessive locus in these five sibs and excluded loci for known white matter disorders, spastic paraplegias and infantile neuroaxonal dystrophy. We found instead linkage for a new chromosomal locus. We conclude that the neurological disorder in this family represents a new type of early onset upper motor neuron disease leading to early selective loss / under-development of pyramidal tracts.

#### P05.101

#### Investigation of cytokine expression pattern associated with the pathogenesis of inflammatory bowel disease

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Inflammation is the shared pathophysiological element for the two main inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC) but its contribution to the triggering and maintaining the pathological condition is still incompletely understood. Our objective was to investigate the pro-inflammatory gene expression profile in colonic mucosa of IBD patients in order to establish correlations with IBD form and disease activity.

RNA was extracted from colonic biopsy samples obtained from IBD patients, after informed consent. The samples were initially screened for inflammatory gene expression profile with a MLPA kit (SALSA R009, MRC Holland) which allows detection of 40 RNA molecules in the same PCR reaction. Subsequently, the selected mRNAs were quantified using RT real-time PCR in individual gene expression assays. We investigated a total of 50 patients with IBD, 33 with CD and 17 with UC. Our preliminary results show an increased expression of some monocyte-derived cytokines (IL1B, IL1RN, IL8, IL12, MIF) and some inflammation associated transcription factors (NFKB, NFKBIA). The cytokines' expression pattern in apparently normal mucosa showed a reduced but still significant inflammatory response.

#### P05.102

#### Regulatory elements at *INS* gene and Neonatal Diabetes

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**Introduction:** Insulin secretion is tightly regulated to maintain blood glucose levels within a narrow physiological range. Recent studies have reported heterozygous missense mutations at *INS* gene in permanent neonatal diabetes (PND). Multiple discrete sequence elements within the proximal promoter region (5'UTR) affect insulin expression, and also the 3'-untranslated (3'-UTR) region has been identified as critical for murine preproinsulin mRNA stability.

**Objective:** To evaluate the putative contribution of *INS* in unrelated subjects with unexplained neonatal diabetes (ND =14).

**Methods:** the 3 exons (including untranslated exon 1), intron-exon junctions and the 5' and 3' untranslated regions of *INS* were PCR amplified with specific primers and direct sequencing was performed.

**Results:** No mutations were identified in the coding sequence. However, we detected 5 novel variants. Three of them were situated in the first exon:

- One patient presented 2 contiguous variants in compound heterozygosis (c.-332C>G)+[-331C>G]).
- One patient carried a heterozygous variant (c.-170A>G).

One patient presented an intronic variant (c.188-31G>A) which was inherited from the father, who also presented with PND.

In a patient with PND a homozygous variant was detected in the 3'UTR (c.\*59A>G). Both parents carried this variant and both presented with glucose intolerance. This variant is situated at the 6<sup>th</sup> nucleotide within in the polyadenylation signal site; (AAAUAA - AAAUAG).

**Conclusions:** Alterations in regulatory elements at *INS* are associated with an earlier onset of PND compared to previously described coding mutations. Mutations in the insulin gene are rare in Spanish patients with neonatal diabetes.

#### P05.103

#### FoxP3 expression on different stage of type 1 diabetes mellitus

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FoxP3 expression is considered as key factor for autoimmunity preventing. It is known that FoxP3 expression level is decreased under type 1 diabetes mellitus. However there no information about the expression level on different stage of this disease.

We investigated FoxP3 expression among for the first time revealed type 1 diabetes mellitus patients (up to 1 year, early disease stage) and a group of patients through 15-25 years after disease beginning (late stage of disease). FoxP3 expression level was determined comparative to expression of GAPDH gene or CD4 gene expression level by Real Time PCR. It was revealed that FoxP3 expression was increased on late stage of disease relatively to early disease stage as with GAPDH so CD4 genes. There were 0,32±0,13 on early disease stage and 1,1±0,35 on late stage for FoxP3 expression level ( $P=0,02$ ). The increasing of FoxP3 expression may mean the decreasing of autoimmune reaction on advanced stage of diabetes. Possibly it reflects equilibrium state of immunity and produced by β-cells antigen.

#### P05.104

#### The influence of the genetic polymorphisms on the transcription factor binding to the IL-4 promoter

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**Background:** Promoter polymorphisms of IL-4 gene C-33T and C-59T are well described. They influence both IL-4 and serum IgE concentrations in a human organism. **Objective:** To evaluate the mechanism of the phenotypic effect of these polymorphisms, specifically to identify the transcription factors (TF) selectively binding to these loci.

**Methods:** Sequences flanking these loci were analyzed *in silico* to

predict the TF binding the selected regions. This prediction was experimentally analyzed by EMSA with nuclear extract of Jurkat T-cell line. The sequences of Cy5-labeled ds oligonucleotides flanked the polymorphic loci and were 39bp (C-33T) and 35bp(C-590T) long. **Results:** It was predicted that TFs Oct-1, CREB, C/EBP and GATA could selectively bind C-33T polymorphism locus while MZF-1, Sox-5, PU.1, AP-2, GR, VDR, E2F-1 and T3R-alpha could bind C-590T locus. We observed that C-33T EMSA probes associated with two specific complexes. The TFs slightly discriminated between -33C and -33T probes. The unlabeled consensus ds oligonucleotides for the Oct-1 completely inhibited the lower shift band. Both bands were not inhibited by the oligonucleotides corresponding to the C/EBP, CREB and GATA binding sites. As for C-590T polymorphism, two specific bands were observed in the sample with -590C probe rather than -590T probe. They were not inhibited by the unlabeled concurrent probes for the predicted TF binding. **Conclusions:** Oct-1 transcription factor could be responsible for the phenotypic effect of C-33T IL-4 promoter polymorphism. The factor responsible for the C-590T polymorphism does exist but should yet be identified. The work was supported by the RFBR grant 08-04-01535.

#### P05.105

##### Effect of Semax treatment on expression of the neurotrophins and their receptors in ischemic rat hippocampus

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Neurotrophins are known as natural neuroprotectors involved in proliferation, differentiation and survival of neuronal and glial cells. We analyzed the effect of synthetic polypeptide Semax (Met-Glu-His-Phe-Pro-Gly-Pro) upon expression of neurotrophins *Bdnf*, *Ngf*, *Nt3* and their receptors *TrkB*, *TrkA*, *TrkC*, *p75* in rat hippocampus 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 or saline 1, 4 and 8 hours after the occlusion. Ischemic rats with saline injection were used as control groups. The mRNA expression of neurotrophins and its receptors was assessed by relative quantification using real-time RT-PCR. Gapdh was used as the reference gene. The neurotrophins' transcription was increased compare with the level of these transcripts in control animals: for *Bdnf* at 1h, 2h and 12h; for *Ngf* at 1h, 12h and 24h and for *Nt3* at 1h and 12h after operation. Under Semax treatment the mRNA expression of neurotrophin's receptors was increased compare with control as well: for *TrkB* at 30 min, 1h and 24h, for *TrkC* at 8h and 12h, and for *p75* at 2h, 4h and 12h after occlusion. The level of *TrkA* mRNA was decreased at 1h, 2h, 4h, and 24h after surgery; at 8h it was increased. It could be suggested that neuroprotective effect of Semax is possibly mediated by neurotrophins and its receptors.

#### P05.106

##### Semax and its C-terminal Pro-Gly-Pro tripeptide change the transcription profile of growth factors and their receptors genes after focal cerebral ischemia in rats

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Neuroprotective polypeptide Semax is used for acute therapy of stroke. The effect of Semax and its C-terminal PGP tripeptide on mRNA expression of 84 genes representing growth factors and their receptors in the lesioned cortex of rat brain after 3 h of permanent middle cerebral artery occlusion (pMCAO) was analyzed using the Rat Neurotrophin and Receptors gene array ("RT<sup>2</sup> Profiler™ PCR Array, Superarray). Under Semax treatment in the lesioned cortex mRNA expression of 19 genes was altered, the change in transcription of 7 genes of these 19 also was observed under ischemic conditions. PGP promoted the transcriptional alteration of 23 genes. The list of genes which mRNA expression was changed under Semax treatment only partly coincided with the list of genes that was changed under PGP treatment; the direction of such transcription alteration was the same. The most marked (two times and more) alteration of mRNA expression under Semax and

PGP treatment was observed for *Lif*, *Galer1*, *TrkA* and *p75*. Some of the genes which mRNA expression were changed by Semax treatment only: *Fos*, *IL10ra*, *Il1b* and *Tgfb1*; and by PGP treatment only: *Fgf2*, *Hctr1*, *Tacr1*, *Ntf5* and *Ptger2*. Hence it was suggested that Semax effects observed earlier do not reflect only PGP activity. Obviously the mechanisms of action of these peptides are not the same and they each have their own specific effects. From the list of genes altered by Semax or PGP two types of growth factor targets were suggested: neural tissue (neurons and glial cells) and vascular cells.

#### P05.107

##### Novel mutations in myoclonin1/EFHC1 in sporadic and familial juvenile myoclonic epilepsy

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#### P05.108

##### Long QT Syndrome: Two Novel Exonic KCNH2 Mutations

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Introduction. K Channels made with the **KCNH2** protein are active in the heart muscle, where they transport potassium ions out of cells. The gene contains 15 exons spanning approximately 19 kb on chromosome 7q35. A feature of K channelopathy is pronounced prolongation of the QT interval and arrhythmia that can lead to sudden death.

Materials and methods. The subjects studied included a 6 members family with 3 out of them involved with LQTS. Genomic DNA was extracted according to a standard method, and then **KCNH2** were PCR-amplified and sequenced for identifying LQTS-causing mutations.

Results. Two exonic novel mutation of **KCNH2** in the exon 6 which was present also both in his mother and his elder sister and also One more novel intronic mutation.

Discussion. The present data, combined with those from previous studies, give more information on KCNH2 mutations which affect loop and pore proteins. The proband , a 16 years old boy with novel muta-

tion in exon 6 (C>T) which converts arginin into lysine . Additionally there was an intronic mutation (C>A) which has not been reported up to this time although predicted to have no effect on protein truncations. In conclusion, this is the first frameshift mutation in Iranian population and we have provided additional data of **KCNH2** mutations in LQTS patients. These findings will contribute to further understanding of the function and structure of **KCNH2** and the phenotype-genotype correlation in hereditary LQTS.

#### P05.109

##### Gene scanning of KCNQ1 and KCNE1 by high-resolution melting analysis

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KCNQ1 encodes the larger subunit (KvLQT1) and KCNE1 the small subunit (MinK) of the I<sub>ks</sub> protein. I<sub>ks</sub> protein is the slowly activating delayed rectifier potassium channel. Mutations in single genes reduce the I<sub>ks</sub> current and cause the similar Long QT Syndrome (LQTS) phenotype. LQTS is a cardiovascular disorder characterized by an abnormality in repolarization, leading to a prolonged QT interval.

KCNQ1 consists of 16 exons and encodes a protein of 676 amino acids. There have been identified 246 mutations in this gene. KCNE1 consists of just 3 exons, encoding a protein of 129 amino acids. Only 30 mutations of this gene have been found.

For the mutation scanning we have used multiplex SSCP with sensitivity about 80 %. Now, we detect the mutations using high-resolution melting analysis. This method is based on PCR in the presence of the double-strand DNA binding dye, and tracking the nucleic acid melting by monitoring the fluorescence of the sample across a defined temperature range. Melting profiles can be used to identify the presence of sequence variation within the amplicon. The method has almost 100% sensitivity and specificity when used on products up to 400bp. We have used this method for detection of 7 described mutations (S225L, T312I, G314S, G325R, T587M, A590T and R591H) in KCNQ1 and two SNPs (S38G and D85N) in KCNE1 with the sensitivity 100 %.

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#### P05.110

##### Identification of mutations in KLK4 genes among Iranian patients with amelogenesis imperfecta

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Amelogenesis imperfecta (AI) refers to a group of inherited tooth disorders characterized by abnormal enamel formation. The incidences of AI vary widely; from 1 in 700 people in northern Sweden to 1 in 14,000 people in the United States. In spite of the fact that many studies have been carried out in different centers on molecular aspects of this disorder, the genetic basis of non-syndromic forms of AI is unknown. So far, four genes have been documented that associated with AI, AMELEX, ENAM, KLK4 and MMP20 genes. We performed molecular genetic studies on 10 Iranian families with different models of inheritances according to pedigree analysis. In this study, three genes including ENAM, KLK4, and MMP20 which account for majority of AI and autosomally inherited were chosen for mutation detection by a polymerase chain reaction (PCR) and single-stranded conformation polymorphism (SSCP). Our results from SSCP revealed genetic alterations in ENAM, MMP20 and KLK4 genes. We found mutations in KLK4 exon 3 in 7 patients. In order to identify the type of mutation, samples were subjected for DNA sequencing. Our findings suggested that the incidences of KLK4 mutations in Iranian population might be higher than other population.

#### P05.111

##### Three novel mutations in the lactase gene (LCT) underlying congenital lactase deficiency (CLD)

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**Background:** Congenital lactase deficiency (CLD) is a severe gastrointestinal disorder of newborns that is inherited as an autosomal recessive trait and is enriched in the isolated Finnish population. The diagnosis is based on clinical symptoms and low lactase activity in intestinal biopsy specimens. Five mutations in the lactase gene (LCT) have so far been identified to underlie CLD.

**Methods:** To search for new mutations underlying CLD we assayed disaccharidase activities in intestinal biopsy specimens and screened the coding region of LCT gene by direct sequencing from one Italian, two Finnish and two Turkish patients with clinical symptoms compatible to CLD.

**Results:** Three novel mutations in the LCT gene were identified. A single nucleotide substitution leading to an amino acid change S688P in exon 7 and a premature stop codon E1612X in exon 12 of the LCT were present in the patient of Italian origin. A novel substitution of R1587H in exon 12 was found in a heterozygous form from one Finnish patient. Both Finnish patients were heterozygous for the Finnish founder mutation Y1370X. Analyses of another Finnish patient are still ongoing. The previously reported missense mutation G1363S in exon 9 was found in a homozygous state in two siblings of Turkish origin. Comparison of clinical phenotype and the location and/or type of a mutation in the LCT gene shows that all mutations lead to a similar phenotype.

**Conclusions:** A total of eight mutations are known in CLD. This is the first report of mutations in non-Finnish patients.

#### P05.112

##### Functional characterization of point mutations in the LDLR gene found in Portuguese patients with clinical diagnosis of familial hypercholesterolaemia.

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The LDL receptor is a surface glycoprotein that mediates binding and uptake of cholesterol-rich lipoproteins from plasma, in particular LDL. Mutations in the *LDLR* cause familial hypercholesterolaemia (FH), which results in defective catabolism of LDL leading to premature atherosclerosis and CHD.

Five missense mutations found in the *LDLR* gene during the "Portuguese FH Study", responsible for protein variants V408L, W469R, S627P, P664S and V838M, were studied in order to assess their pathogenicity.

The different *LDLR* mutants were generated by site-directed mutagenesis and expressed in CHO-IdlA7 cells lacking endogenous expression of *LDLR*. To determine the effects of mutations on *LDLR* function we measured saturable binding plus internalization and degradation of <sup>125</sup>I-labelled LDL at 37°C and estimated mature *LDLR* at cell surface by immunoblotting.

All mutant constructs resulted in production of a detectable mature protein in CHO-A7 cells, except variant W469R which accumulated the precursor form. Variants W469R and V408L were severely impaired in their ability to mediate uptake and degradation of <sup>125</sup>I-LDL and showed reduced amounts of *LDLR* at cell surface. Variant S627P retained ~40% and P664S ~60% of normal *LDLR* activity. V838M variant showed essentially the same activity as the wild-type *LDLR*.

Results suggest that four of the variants studied are mutations causing disease in patients carrying those alterations and V838M is a rare non-pathogenic variant. The severe effect that V408L mutation has in *LDLR* function does not correlate with the patient's phenotype, suggesting other genetic or/and environmental factors may be involved in phenotype modulation.

#### P05.113

##### Genetic testing for Leber Congenital Amaurosis (LCA): a 3-year experience

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LCA is genetically highly heterogeneous with the involvement of large disease genes, which hamper genetic testing. The purpose of this study was to determine the prevalence of mutations in 6 common LCA genes in 108 LCA patients, mainly of Belgian origin, in order to optimize a genetic screening strategy for LCA.

First, LCA chip screening revealed a mutation in 23% of all patients. Second, direct sequencing of AIPL1, CRB1, CRX, GUCY2D, and RPE65 revealed causal mutations in 4.6%. Third, we performed targeted mutation analysis of the CEP290 mutation c.2991+1655A>G. We found this mutation in both homozygous (2/108) and heterozygous (16/108) state. A second mutation was identified through sequencing of the total coding region. Subsequently, the remaining patients were screened for 4 additional recurrent CEP290 mutations. p.Lys1575X was found in 3.7% and c.[3310-1G>A;3310C>A] in 0.9%, demonstrating that c.2991+1655A>G is not present in all CEP290-related LCA cases. A second mutation was identified in 3 cases; cDNA sequencing is ongoing in the other ones. Finally, sequencing of the total coding region of CEP290 is being performed in the remaining 39 cases. So far, this revealed a homozygous mutation in one case.

In conclusion, we found mutations in 50% of all patients (22% in CEP290; 15% in CRB1; 6% in RPE65; 3% in AIPL1; 2% in CRX and 2% in GUCY2D). A combined genetic testing strategy consisting of LCA chip analysis and targeted mutation screening of 5 recurrent CEP290 mutations, represented an efficient first-pass screening, revealing causal mutations in 44.4% of our LCA population.

#### P05.114

##### Genetic study of Tunisian patients with Leigh syndrome: presence of the T8993G mutation in the MT-ATP6 gene and 2 new mutations in the MT-ND2 and MT-ATP8 genes

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Leigh syndrome (LS) is a genetically heterogeneous, neurodegenerative disorder that predominantly affects children in early infancy or childhood and leads to death within months or years. This disorder is characterized by necrotic lesions in the brainstem, basal ganglia, and thalamus. The symptoms are variable, but in most cases include psychomotor retardation, optic atrophy, ataxia, dystonia, failure to thrive, vomiting, seizures, and respiratory failure. Mutations causing Leigh syndrome have been found in both mitochondrial and nuclear DNA. Most of the described mitochondrial mutations were in ND3 and ND5 and a few ones were in ND6 and ND4. In addition, there are single case reports of mutations in synthetic genes such as tRNA<sup>Trp</sup> and tRNA<sup>Lys</sup> genes. In the present study, we carried out a systematic sequence analysis of mitochondrially encoded complex I subunits: ND2, ND3, ND4, ND5 and ND6 in 16 Tunisian patients with Leigh syndrome. We also performed a sequence analysis of the mitochondrial ATPase 6, tRNA<sup>Ala</sup>, tRNA<sup>Leu(UUR)</sup>, tRNA<sup>Trp</sup> and tRNA<sup>Lys</sup> genes in these patients. Mitochondrial DNA sequencing of these genes revealed the presence of the T8993G mutation in the ATPase 6 gene in 1 patient belonging to a Tunisian family with Maternally Inherited Leigh Syndrome (MILS). In this family, we also found 3 new mutations responsible for aminoacid changes in a highly conserved region of the MT-ATP 6 protein. In addition, we detected 60 known polymorphisms, 19 new nucleotide variants and 2 novel mutations in 2 patients with Leigh syndrome in the ND2 and in the ATPase 8 gene.

#### P05.115

##### A novel mutation of the lipoprotein lipase gene associated with childhood hypertriglyceridaemia

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Familial LPL deficiency is a rare autosomal recessive disorder that affects about 1/1.000.000 children. Patients with classical lipoprotein

lipase deficiency present in the first several months of life with masked hypertriglyceridaemia, often ranging between 5000 to 10.000 mg/dl. Hypertriglyceridaemia caused by decreased or absent Lipoprotein lipase (LPL) activity is associated with increased blood lipoproteins. LPL plays a functional role in regulation of the lipoproteins. To date, approximately 140 mutations have been identified within LPL gene in human genome. Most of the mutations are located within the coding region of this gene. We performed DNA sequencing of 9 exonic regions of LPL gene including promotor region of 10 patients with hypertriglyceridaemia. We detected one novel homozygous missense mutation that alters aminoacid at position 221 (I221T). The homozygous mutation causes the substitution of Isoleucine to Threonine at codon 221 in exon 5. We evaluated the characteristic of patients, laboratory findings and associated disorders. Treatment of patients with LPL deficiency is difficult. Therefore, detection of mutations might be useful for better therapy and prenatal diagnosis of the patients.

#### P05.116

##### Detección and characterization of large rearrangements in the SLC7A7 gene in Lysinuric Protein Intolerance patients

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Lysinuric protein intolerance (LPI) or hyperdibasic aminoaciduria type 2 (OMIM 222700) is a rare autosomal inherited disease, caused by defective cationic amino acid transport 4F2hc/y<sup>+</sup>LAT-1 at the basolateral membrane of epithelial cells in the intestine and kidney. LPI is a multi-systemic disease with a variety of clinical symptoms, such as osteoporosis, hypotonia, growth delay, pulmonar insufficiency, or renal insufficiency. LPI is diagnosed by the presence of excessive urine excretion of dibasic amino acids, especially lysine, and by their poor intestinal absorption that leads to low plasma levels of dibasic amino acids. The SLC7A7 gene, which encodes the y<sup>+</sup>LAT-1 protein, is mutated in LPI patients. Mutation analysis of the two promoters and all exons of the SLC7A7 gene was performed in ten patients from nine unrelated LPI families from different ethnic backgrounds. Point mutation screening was performed by exon direct sequencing and a new multiplex ligation probe amplification (MLPA) assay was set up for large rearrangement analysis. Eleven SLC7A7 specific-mutations were identified in these patients. Two out of seven novel mutations were large rearrangements of the SLC7A7 gene.

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#### P05.117

##### Our methodical process of identification of Malignant hyperthermia causal RYR1 mutations

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Our work is focused on performing an efective and relative inexpensive molecular genetic diagnostic assay of malignit hyperthermia (MH), life-threatening and frequently fatal disorder triggered by commonly used anesthetics. Susceptibility to MH (MHS), dominantly inherited predisposition to MH, is diagnosed by using an invasive diagnostic test on excised muscle bundles, the in vitro contracture test (IVCT). An alternative diagnostic test to IVCT is mutation analysis of the ryanodine receptor (RYR1) gene. RYR1 is an essential component of the calcium homeostasis of muscles in mammals. Defects in the RYR1 gene in humans (19q13.1) are associated with MH. Until now, 29 RYR1 mutations causing MH have been listed by European MH Group. A detection of MH causative RYR1 mutations can be used as predictive genetic testing. Our aim is to develop sufficient, effective and fast mutation-detection procedures aimed at direct detection of MH causative RYR1 mutations. To date we established molecular diagnostic assays to detect RYR1 mutations using screening metods. Sequencing the

entire RYR1 complementary DNA (cDNA) and melting point analysis of fluorescently labelled probes after high speed PCR amplification on real-time enabled us to confirm MH diagnosis on molecular level in 50 MHS individuals. Recently we establish High-resolution melting (HRM) as a method that allows RYR1 mutation scanning and genotyping. We expect that our methodical process of identification of MH causal mutations in MHS patients and subsequent performing noninvasive predictive genetic testing in their family members decrease number of individuals which would have to undergo the invasive IVCT.

#### P05.118

#### Mutation screening of *FBN1* and *TGFBR2* genes in patients with Marfan and marfan-like syndromes from Russia: 4 mutations and 11 polymorphisms have been found

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Marfan syndrome (MFS) is an inherited autosomal dominant connective tissue disorder. Abnormalities appear in skeletal, ocular and cardiovascular systems. The main cause of MFS is mutations in the fibrillin1 gene (*FBN1*). Recently, the transforming growth factor beta receptor 2 gene (*TGFBR2*) has been shown to be associated with a second type of this disorder with typically mild or absent ocular involvement (MFS type 2) as well as with classical MFS. We analyzed 30 exons of *FBN1* gene and 4 exons of *TGFBR2* gene in 80 patients with MS and marfan-like syndromes from different regions of Russia. SSCP analysis revealed different abnormal migrating patterns. We identified two missense mutations (G1176Y in 28 exon and C2489Y in 60 exon) which affects cbEGF-like motifs of fibrillin-1 protein in two patients with classical MFS symptoms. We also found 9 polymorphisms both in coding and non coding regions of *FBN1* gene, five of them are not previously described. One novel mutation (c.670C>T; T223M) has been found in *TGFBR2* gene in two unrelated patients with marfan-like syndrome who did not fulfill Ghent nosology and who did not have mutations in *FBN1* gene. Mutation T223M affects highly conserved serine/threonine protein kinases catalytic domain that leads to change phospho transferring status of *TGFBR2* protein. In addition two novel polymorphisms have been found in intronic regions of *TGFBR2* gene. Mutation screening of *FBN1* and *TGFBR2* genes continues.

#### P05.119

#### Novel mutations of *FBN1* in Czech population

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Marfan syndrome (MFS) is a heritable autosomal dominant disorder of connective tissue with prevalence of between 1 in 5-10 000. Approximately 25% of MFS patients are sporadic cases due to new mutations. MFS is noteworthy for its clinical variability. Major features of the MFS include cardiovascular disorders (dilatation and dissection of ascending aorta), eye disorders - ectopia lentis, defects of skeletal system - pectus carinatum, pectus excavatum and/or other diagnostics criteria as arachnodactyly.

MFS is caused by mutations in fibrillin 1 gene (*FBN1*) resulting in defective glycoprotein fibrillin-1. *FBN1* is located on chromosome 15 at locus q15-q21.1. Recently, there are showed that two other genes *FBN2* (5q23-q31) and *TGFBR2* (3p22) influence MFS.

Since 2006 we have done a molecular analysis for MFS diagnosis in Czech Republic. The molecular analysis includes mlpa (multiplex ligation-dependent probe amplification), separation of PCR products by SSCP (single-strand conformation polymorphism) and sequencing. We have performed mutation detection on 150 patients with suspected MFS. There were detected 20 novel mutations.

#### P05.120

#### Large deletions account for a significant fraction of mutations in Marfan syndrome

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Marfan syndrome (MFS) is an autosomal dominant hereditary disorder of connective tissue caused by mutations in the *FBN1* gene. Cardinal manifestations include proximal aortic aneurysm, ectopia lentis, and involvement of the skeletal system. About 60 to 90% of the cases with clinically diagnosed Marfan syndrome can be explained by a *FBN1*

mutation. This rate can be raised by subsequent sequencing analysis of the genes *TGFB1* and *TGFB2*.

We tested 45 patients without identified mutation in the three genes by use of MLPA (multiplex ligation-dependent probe amplification) and investigated whether large deletions in the *FBN1* gene would increase the mutation detection rate sufficiently for incorporation of MLPA in the routine diagnostics of Marfan syndrome. The patients fulfilled or partially fulfilled the diagnostic criteria (Ghent nosology).

We identified three large deletions in the 3' region of the *FBN1* gene; ranging from exon 50 to 54, exon 55 to 58 and exon 58 to 63 (the last one was previously described by Singh et al., J. Mol. Cell. Cardiol. 2007). A fourth deletion comprised the complete *FBN1* gene. Breakpoints of the deletions were determined by long-range PCR techniques. Implications for the resulting protein product, as well as the phenotypes of the patients, will be discussed.

We estimated a deletion detection rate of 9% in this pre-screened patient group, corresponding to an overall rate of 3% among all patients with MFS. These data imply an inclusion of MLPA analysis in the routine diagnostics of Marfan syndrome.

#### P05.121

#### Alternative splicing variants of MCPH1 with distinct functions

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Primary microcephaly (MCPH) is an autosomal recessive disorder characterized by pronounced reduction of brain size and variable mental retardation without additional neurological deficits. Four underlying genes have been identified to date. Hallmark of patients with mutations in the MCPH1 gene (MIM# 606858; MIM# 607117) is a cellular phenotype of premature chromosome condensation, PCC, in the G2 phase and delayed decondensation in G1. MCPH1 is located on human chromosome 8p23.1, consists of 14 exons, and encodes the protein microcephalin containing one N-terminal and two C-terminal BRCT-domains as well as an NLS sequence.

RT-PCR showed alternatively spliced variants of MCPH1-mRNA. In addition to the full length MCPH1 (MCPH1-L), we detected a variant skipping exon 8 (MCPH1-S) and another without exons 9-14 (MCPH1-B). The resulting polypeptides are lacking the NLS or the two C-terminal BRCT-domains, respectively.

Expression of any of the three variants as GFP-fusion proteins by retroviral transfer resulted in complementation of the PCC phenotype in MCPH1-deficient cells. In contrast, a construct containing MCPH1 without the N-terminal BRCT-domain (MCPH1Δ1-7) did not complement the PCC phenotype, suggesting a role of this domain in regulating chromosome condensation, presumably through interaction with condensin II.

The fluorescent signals confirmed nuclear localization of all three variants. For MCPH1-S a GFP-signal was detected in the cytoplasm as well, which appeared to co-localize with the centrosomes, whilst MCPH1-L and MCPH1-B re-localized to chromatin during anaphase. In addition, variants containing C-terminal BRCT domains re-localized to gamma-H2AX foci indicating participation of these variants in DNA-damage responses.

#### P05.122

#### Investigation of six mental retardation loci (MCPH1, MCPH2, MCPH3, MCPH4, MCPH5, and MCPH6) associated with microcephaly in northeast & southeast of Iran

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Primary autosomal recessive microcephaly is defined as a reduction in head circumference. Six out of ten Non-Syndromic Autosomal Recessive Mental Retardation (NS-ARMR) loci associated with microcephaly (MCPH1-MCPH6) and belong to the family of MCPH (autosomal re-

cessive primary microcephaly). So far four genes have been identified: *MCPH1*, encoding Microcephalin; *MCPH3*, encoding CDK5RAP2; *MCPH5*, encoding ASPM, and *MCPH6*, encoding CENPJ. *MCPH5* and *MCPH1* are the most common loci based on MCPH heterogeneity studies in Pakistani and Indian populations. The objective of this study was to investigate prevalence of ARMR associated with microcephaly in Iranian families from northeast & southeast of Iran. A total of 20 consanguineous families with two or more affected individuals with ARMR inheritance pattern have been collected after obtaining consent form. Clinical examination and exclusion of chromosomal abnormalities of the families were completed and followed by homozygosity mapping using STRs (Short Tandem Repeats) markers for six mentioned MCPH loci. Sequencing was performed for the linked families. Nine out of twenty families were linked to four of these loci as follows: Five were linked to *MCPH5* (25%), and *MCPH1*, *MCPH2* were linked to one family each (5%), and two were linked to *MCPH6* (10%). We have been able to identify four novel mutations in four families of the *MCPH5*. According to our findings *MCPH5* seems to be the most prevalent loci in Iranian families with mental retardation and microcephaly. Sequencing for the other linked families is currently underway.

#### P05.123

#### MicroRNA expression and post-transcriptional target genes analysis from one pair of monozygotic twins discordant for trisomy 21: understanding phenotypic variability in DS patients

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The understanding of the molecular pathogenesis of trisomy 21 (T21) is remarkably poor since it is not clear how the extra copy of HSA21 leads to a wide range of phenotypes. We investigated a new class of functional sequences: microRNAs (miRNAs), that act primarily as post-transcriptional repressors of target genes through 3'UTR interactions. We studied microRNA gene variation on primary fibroblasts and heart tissue from one pair of monozygotic twins discordant for T21. Expression was assayed using Taqman qRT-PCR on 365 mature miRNAs, 4 of which are encoded by HSA21. Most of deregulated microRNAs are tissue specific. Around 12% (fibroblasts) and 25% (heart) of microRNAs show a statistically significant difference between the T21 and normal twin, with an average up-regulation of 1.6fold and down-regulation of 0.6fold. Expression levels revealed that HSA21-miR-155 and HSA21-miR-99a are up-regulated about 2fold in fibroblasts and heart tissue, respectively. We have also examined transcripts that are post-transcriptionally deregulated in T21 and potential target genes of those deregulated microRNAs. To do so, we separated RNA molecules of primary fibroblasts from the same pair of twin in a sucrose gradient according to their association with ribosomes. Pools of fractions corresponding to the heavy ribosomal fraction (translated RNA) and total RNA were hybridized to a gene expression microarray. We identified 45 and 63 transcripts with a post-transcriptional up-regulation and downregulation in the T21 twin, respectively. To complete this study, we are currently looking for predicted miRNA target genes and correlated miRNA expression with the expression of its target genes.

#### P05.124

#### Distinctive patterns of miRNA expression in human muscular disorders

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The muscular disorders are a heterogeneous group of inherited diseases characterized by muscle wasting and progressive weakness, resulting in significant morbidity and disability. Although considerable progress has been made in the understanding of these disorders, the underlying molecular pathways remain poorly understood. In light of their involvement in modulating cellular phenotypes we hypothesized that miRNAs might be involved in the regulation of the pathological pathways leading to muscle dysfunction. We describe a comprehensive miRNA expression profile aiming to identify new elements involved in the regulatory networks of muscle and the signature pattern of 185 miRNAs associated with ten common myopathological conditions in human. While five miRNAs were found to be consistently dysregulated

in all samples analyzed suggesting that these miRNAs are involved in a common underlying regulatory pathway among all diseases, other miRNAs were identified to be dysregulated only in one given disease and not in any of the others thus pointing to their involvement in a unique regulatory mechanism.

The subsequent identification of potential target genes and the unraveling of biological signaling pathways involved in this regulatory level in these disorders, point to an additional dimension of regulation of muscle function mediated by miRNAs. Together with the tight post transcriptional regulation at the mRNA level identified in Duchenne and Miyoshi myopathy and specific mRNA:miRNA predicted interactions, some of which are directly involved in compensatory secondary response functions and others in muscle regeneration, these findings suggest an important role of miRNAs in the pathology of muscular dystrophy.

#### P05.125

#### Multiple Sclerosis Disease and Mitochondria

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Multiple Sclerosis (MS) is a multifocal demyelinating central nervous system disorder. To assess relationship between mtDNA haplogroups and MS, we have sequenced the mtDNA HVS-I in 54 MS patients and 100 control subjects. In this study, kinetic analysis of mitochondrial respiratory chain complex I enzyme was performed on intact mitochondria isolated from fresh skeletal muscle in MS patients ( $n = 10$ ) and control subjects ( $n = 11$ ). The frequencies of the Asian (M, BM) and European (N, J, K) mtDNA haplogroups in five major regions of Iran was investigated. Unexpectedly, the frequencies of the Asian haplogroups M and BM were low in Iran (2.34% for haplogroup M; 17.6% for haplogroup BM and 80.06% for haplogroup N).

We have found that haplogroups A and K are significantly more abundant in MS patients ( $P=0.042$  for haplogroup A and  $P=0.0005$  for haplogroup K). Our findings showed that complex I activities were significantly reduced ( $P=0.007$ ) in patients compared with control. However, we could not find deletion in mtDNA of patients with MS. Our results revealed that 15 (75%) out of 20 MS patients had point mutations. This study suggested that point mutation occurred in mtDNA might be involved in pathogenesis of MS. Our data suggest that Iranian tribes probably played a remarkable role in the formation of these ethnic groups. It gives the indication that the haplogroup J may be older than 6000-10000 years, and probably developed in Iran, and then expanded to different regions in Europe and Northwest Asia.

#### P05.126

#### The coexistence of an East-Asian mitochondrial anthropological marker and the C8270T, A8332C, and A8347C mtDNA mutations in a Hungarian family with dystonia and juvenile stroke syndrome

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The high variability of the mitochondrial genome contributes to the phenotype of the mtDNA-related disorders. MtDNA mutations have been associated with a variety of clinical manifestations. The 9-bp deletion between mtDNA 8271-8280 was originally thought to be an anthropological marker for peoples of East-Asian origin.

The proband was investigated for marked dystonic features. The clinical symptoms started after a long lasting episode of fever and elevated serum ammonia and lactate level. His brother had transient hyperkinesia, right hemiparesis. Their mother had in her childhood a stroke with aphasia and right hemiparesis. Presently she has moderate truncal ataxia, hypoacusis and cognitive dysfunction.

The mtDNA analysis of the proband found a 9bp deletion "CCCCCTCA" in the mtDNA at the position nt8271-8280, and one C8270T substitution between COII and tRNA<sup>Lys</sup> genes in the non-coding hypervariable segment, and two SNPs (A8332G and A8347C) in the tRNA<sup>Lys</sup> gene. The C8270T SNP is a pathogenic mutation, the A8332G and A8347C SNPs are not described in the literature. None of the three SNP was found in our 100 control cases. The affected Hungarian family belongs to an ancient B mitochondrial haplogroup.

Conclusion: The B haplogroup and the 9 bp small deletion indicate the east-Asian origin of this family which could be explained by the Hungarian history. The 9 bp deletion may serve as a susceptibility factor for further mtDNA alterations. We assume that the clinical symptoms are related to the pathogenic C8270T mutation and the coexistences of the A8347C and A8332G mutations may modify the clinical symptoms.

#### P05.127

#### Mitochondrial nonsyndromic hearing impairment: the Spanish exception?

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Nonsyndromic sensorineural hearing impairment (NSHI) can be caused by few mitochondrial mutations. Among these mtDNA mutations, A1555G mutation in the 12S rRNA gene has been worldy reported and is associated with NSHI maternally transmitted or with aminoglycoside-induced NSHI. A high frequency of A1555G was observed in Spain.

From a large cohort of NSHI patients studied in the National Reference Center for Genetic Deafness in Paris, France, we selected 156 unrelated patients with NSHI maternally inherited and 124 sporadic NSHI exposed to aminoglycosides before hearing loss onset. A1555G was studied by PCR-restriction enzyme analysis and confirmed by sequencing. Absence of GJB2 mutations and GJB6 deletions was verified.

A1555G mutation was observed in 7 of the 156 NSHI familial cases (4,5%) and in 0 of the 124 sporadic cases. The 7 families with A1555G originated respectively from China, Martinique, Arab/Israel, Portugal, Iran, Madagascar and Spain. Homoplasmic A1555G mutation was detected in all the descendants of the oldest studied mother. The HI was present in 51 of the 102 maternal relatives. The calculated penetrance of A1555G in this study is 50%. The HI was very heterogeneous with an onset ranging from 0 to 30 years and a severity varying from mild to profound.

This study shows that the frequency of A1555G in NSHI maternally inherited is lower in France than in Spain (4,5% versus 61%) and in sporadic cases (0 versus 3%). This reported variability could be due to the different use of aminoglycoside antibiotics in the two countries or to genetics factors.

#### P05.128

#### Mitochondrial ATPase 6 gene as a hot spot for Neurodegenerative disorders?

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The mitochondrial DNA (mtDNA) may play an essential role in the pathogenesis of the respiratory chain complex activities in neurodegenerative disorders such as Huntington's disease (HD), Spinocerebellar Ataxias (SCA), and Multiple Sclerosis (MS), Ataxia-Telangiectasia (AT), Friedreich's Ataxia (FA) and Duchene Muscular Dystrophy (DMD) as neuromuscular disease. Dysfunction of the mitochondrial Respiratory Chain (RC) has been shown in patients with neurological disorders. The tRNA genes mutations are one of hot spots that cause mitochondrial disorders.

To determine mtDNA damage, we investigated deletions based in four areas of mitochondrial DNA, in a group of 120 patients clinically diagnosed with one of mentioned neurological disorders. We screened for *tRNA<sup>Leu</sup>*, *tRNA<sup>Lys</sup>*, *COII*, *ATPase6/8* and *ND1* genes of mtDNA of patients with HD, SCA, MS, AT, FA and DMD.

Results showed that ATPase 6 gene is a hot spot region rather than other regions. Several new nucleotide variations resulting in amino acid changes were observed in patients that altered protein structure regarding to essential changes in amino acids.

The secondary mitochondrial respiratory chain defects encountered in these disorders can also be explained by mitochondrial that degenerate either as part of a more widespread cellular insult, or as a consequence of a different specific defect. Most mitochondrial proteins have to be imported from the cytoplasm and directed to the mitochondria. So this study suggests that the high rate of mutations in ATPase 6

could be result in interaction of nuclear protein with mtDNA genome.

#### P05.129

#### Screening for MELAS mutations in Italian patients having stroke-like episodes

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Mitochondrial encephalomyopathies are a multisystemic group of disorders that are characterised by a wide range of clinical, biochemical, and genetic mitochondrial defects. Among this group of disorders, the Mitochondrial myopathy, Encephalopathy, Lactic Acidosis with Stroke-like episodes (MELAS) syndrome is one of the most frequently occurring maternally inherited mitochondrial disorders. The age of onset is highly variable and it is very rare in patients over the age of 40. Mutations in tRNA, especially A3243G in the *MTTL1* (*tRNA<sup>Leu</sup>*) gene, are accounted for more than 80% of the cases and other mtDNA mutations such as T3271C have also been described. We analyzed 55 Italian patients clinically exhibiting stroke-like episodes, migraine, ataxia and dementia for the two primary mutations mentioned above in the *MT-TL1* gene. This molecular investigation was carried out using PCR-RFLP with the restriction endonuclease Apa I and Afl II respectively. None of the patients or the 25 controls subjects, matched for ethnic background, gender and age, were found to carry these mutations (A3243G and T3271C) in homoplasmic/heteroplasmic form. However, after direct sequencing of the *MT-TL1* gene and a part of the *MT-ND1* gene, we no detected mt-mutations in the tested patients and controls and we confirmed that MELAS is an extremely rare disease. Moreover, we also found a A3480G synonymous variation in the *MT-ND1* in a 51-year-old man, whose medical history was significant for ischemic stroke of undetermined origin and increase in lactate level in blood.

#### P05.130

#### Two novel mitochondrial DNA mutations in muscle tissue of a patient with limb-girdle myopathy

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Patients with an oxidative phosphorylation (OXPHOS) disorder often present with complex clinical features, including neurological and muscular dysfunction. Mutations underlying these diseases are located in the mitochondrial or nuclear genome. Here, we report on a forty-eight-year-old woman suffering from mild limb girdle myopathy. The enzymatic activities of the OXPHOS enzymes in skeletal muscle tissue of the patient were within the control ranges. However, activity staining following BN-PAGE showed a decreased complex III activity and the presence of complex V subcomplexes. Immunocytochemistry demonstrated a mosaic staining pattern in a minority (about 20%) of the muscle fibers for complex I (subunit 20kd) and complex IV (sub-unit I). Molecular analyses identified two novel heteroplasmic mitochondrial DNA nucleotide aberrations in muscle tissue : an insertion, m.5888insA in the *mt-tRNA<sup>Tyr</sup>* gene and an alteration, m.14639A>G, changing a leucine into a serine residue in the ND6 gene. Less than 1% of both mutant alterations were present in the patient's fibroblasts, while they were undetectable in her blood and in blood and fibroblasts from her mother.

Single muscle fiber analyses clearly demonstrated that COX-deficient fibers, as compared to COX-positive fibers, harbored a significantly higher level of both mtDNA mutations. These results, together with previously defined canonical criteria determining the pathogenic character of mtDNA analyses, suggest that both nucleotide changes are pathogenic mutations.

**P05.131****A novel splicing mutation in the HNF1a gene in an Italian family with MODY3 disease**A. Cappelli<sup>1,2</sup>, S. Silvestri<sup>3</sup>, P. Staffolani<sup>4</sup>, A. Consoli<sup>4</sup>, L. Pianese<sup>1</sup>;<sup>1</sup>Laboratorio Medicina Molecolare, ASUR ZT13, Ascoli Piceno, Italy, <sup>2</sup>School of Advanced Studies: Environmental Sciences and Public Health, Camerino, Italy,<sup>3</sup>Scuola di Specializzazione in Biochimica Clinica, Camerino, Italy, <sup>4</sup>Università degli Studi "G. d'Annunzio", Chieti, Italy.

Maturity-onset diabetes of the young (MODY) is a monogenic form of diabetes mellitus characterized by autosomal dominant inheritance, early age of onset and a primary insulin secretion defect. More than 200 different mutations in the hepatocyte nuclear factor 1a (HNF1a) gene have been shown to cause a common type of MODY, called MODY3. Mutations span the entire gene and most types of mutations have been described; e.g. insertion/deletion mutations, missense mutations, nonsense mutations and splice site mutations. Recently, partial and whole gene deletion mutations also were described.

In the present study we screened for mutation the HNF1a gene in a proband which fulfilled the criteria for MODY3, using direct sequence. The examination was extended to the proband's family: an affected father and unaffected mother and sister.

Here we report the identification of a novel HNF1a mutation at conserved splice acceptor site of exon 5 (IVS4nt-1 G>T) that cosegregated with diabetes in the family. Using a neural network based program, this mutation might be expected to result in the skipping of the exon immediately 3' to the mutation and the utilization of the next available AG site for exon 6. Alternatively, a cryptic AG splice acceptor site in intron 5, at -75 bp 5' to the natural site, could be recruited resulting in inclusion of some intronic sequence. However, truncation of the protein results in both cases. Examination of mutant mRNA transcript will be necessary in order to assess the precise consequence of this mutation.

**P05.132****Contribution of the dHPLC to the diagnosis of VHL somatic mosaicism**S. Lefebvre<sup>1</sup>, C. Gressier<sup>1</sup>, P. Delobre<sup>1</sup>, N. Burnichon<sup>2,3</sup>, S. Pinson<sup>1</sup>, A. Gimenez-Roqueplo<sup>2,3</sup>, R. Salomon<sup>4</sup>, S. Richard<sup>5,6</sup>, A. Calender<sup>1</sup>, S. Giraud<sup>1</sup>;<sup>1</sup>Hospices civils de Lyon, Hôpital Edouard Herriot, Service de génétique, Lyon, France, <sup>2</sup>Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Département de génétique, Paris, France, <sup>3</sup>Université Paris Descartes, Faculté de médecine, Paris, France, <sup>4</sup>Assistance Publique-Hôpitaux de Paris, Hôpital Necker, Service de Néphrologie pédiatrique, Paris, France, <sup>5</sup>Faculté de médecine Paris-Sud, Service de génétique oncologique EPHE, Paris, France, <sup>6</sup>Institut Gustave Roussy, UMR8125, Villejuif, France.

Detection and characterization of somatic mosaicism are difficult because of the low frequency of mutant alleles. We report here the diagnosis of two cases of VHL somatic mosaicism identified by dHPLC. The Von Hippel Lindau disease (VHL) is an autosomic dominant inherited syndrome, predisposing to the development of various tumours, notably retina hemangioblastomas and phaeochromocytomas. Analysis for constitutional change of the VHL gene is realized by sequencing and QMPSF. For two patients addressed for VHL suspicion, the sequencing did not show mutation. However for each patient, on two different frequent change sites, second peaks (respectively c.482G>A and c.500G>C) were discriminated but their heights were small (1/5 of the normal peak) and difficult to differentiate from the usual background noise.

We analyzed these two patients by dHPLC. For each patient, aberrant profile was observed, similar but reduced relative to profile with known mutation at the same site.

With dHPLC collector, we collected and sequenced separately the various peaks. We could clearly observe a superposition of two nucleotides in equivalent heights, which allowed us to confirm the VHL somatic mosaicism.

These two patients need follow-up like other VHL patients even if they have not presented all the characteristic attacks of this syndrome.

We began a retrospective work by dHPLC on the patients addressed for a suspicion of VHL disease, with early or multiple attacks and without identified mutation.

**P05.133****Interaction between MTHFR polymorphisms and development of myopathyc process (hypothesis approbation)**V. C. Sacara<sup>1</sup>, E. V. Scvortova<sup>2</sup>;<sup>1</sup>Centre of reproductive health and medical genetics, Chisinau, Republic of Moldova, <sup>2</sup>Moldavian State University, Chisinau, Republic of Moldova.

**Background:** Polymorphisms of the MTHFR gene can influence the methionine metabolic pathway and folate metabolism. The methylation process is essential for the regulation of gene expression controlling the development and function of the muscles. We noted that DMD patients with the same deletion in the dystrophine gene had different clinical features and severity of pathology process. The main conception of our hypothesis is that exist an interaction between MTHFR polymorphisms and development of myopathyc process.

**Methods:** We analyzed DNA from a case-control study in the RM of 50 DMD probands and 114 controls. MTHFR variant alleles were determined by a PCR-RFLP. Control group data is taken from publication Skibola *et al.*, 1999. The genotyping protocol for the detection of the MTHFR C677T and A1298C polymorphisms were adapted from MG-Center, Moscow (prof. Polyakov A.). Statistic analyses were performed by using SISA.

**Results:** We found the MTHFR C677C allele present among 3(6%)DMD and 61(53.5%) controls ( $\chi^2=32.9, P=0.00$ ), the C677T genotype among 27(54%)DMD, 39(34.2%) controls ( $\chi^2=5.66, P=0.02$ ), and the T677T allele 20(40%)DMD, 14(12.3%) controls ( $\chi^2=16.25, P=0.00$ ). For MTHFR 1298, the A1298A genotype was observed in 33(66%) of DMD, 49(43.0%) of the controls ( $\chi^2=7.37, P=0.00$ ), the A1298C allelic variant was observed in 11(22%) DMD, 54(47.4%) controls ( $\chi^2=9.35, P=0.00$ ), and the rarer C1298C variant was observed among 6(12%) case and 11(9.6%) controls ( $\chi^2=0.21, P=0.65$ ).

**Conclusions:** According to our data were observed statistically significant differences between DMD and control MTHFR genotypes, except C1298C. The MTHFR T677T genotype was higher among DMD patients as we know this mutation leads to reduced MTHFR activity and influence on myopathyc process.

**P05.134****Mutational analysis of the MTHFR gene in four patients with homocystinuria due to severe MTHFR deficiency**R. Urreizti<sup>1,2</sup>, U. Fanhoe<sup>1</sup>, A. Langkilde<sup>1</sup>, C. Esteves<sup>1</sup>, M. Cozar<sup>1,2</sup>, M. Vilaseca<sup>3,2</sup>, R. Artuch<sup>3,2</sup>, A. Baldellou<sup>4</sup>, L. Vilarinho<sup>5</sup>, B. Fowler<sup>6</sup>, A. Ribes<sup>7,2</sup>, D. R. Grinberg<sup>1,2</sup>, S. Balcells<sup>1,2</sup>;<sup>1</sup>Dpt de Genetica, IBUB, Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Centro de Investigacion Biomedica en Red de Enfermedades Raras (CIBERER), ISCIII, Barcelona, Spain, <sup>3</sup>Department of Bioquímica Clínica, Hospital Sant Joan de Deu, Barcelona, Spain, <sup>4</sup>Unidad de Enfermedades Metabólicas, Hospital Infantil Miguel Servet, Zaragoza, Spain, <sup>5</sup>Instituto de Genética Médica Jacinto Magalhães, Porto, Portugal, <sup>6</sup>Metabolic Unit, University Children's Hospital, Basel, Switzerland, <sup>7</sup>Divisió d'Errors Congènits del Metabolisme (IBC), Departament de Bioquímica i Genètica Molecular, Hospital Clinic, Barcelona, Spain.

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which acts as a methyl donor in the remethylation of homocysteine to methionine. Disruption of MTHFR activity results in severe hyperhomocysteinemia and causes vascular and neurological disorders and developmental delay. Four patients with severe hyperhomocysteinemia and hypomethioninemia were examined with respect to their symptoms, their MTHFR enzyme activity and their genotypes at the MTHFR gene. We found three novel mutations: two missense mutations c.664G>T (p.V218L) and c.1316T>C (p.F435S), and a one bp deletion c.1768delC (p.L590C fs X70). We also found c.1420G>T (p.E470X), a previously reported nonsense mutation. Four new genotypes were identified: Patient 24 was homozygous for p.V218L and the common polymorphism p.A222V (c.667C>T); patient 73 was homozygous for p.E470X; patient 86 was homozygous for p.F435S and the common polymorphism p.E429A (c.1298A>C); and patient 95 was homozygous for c.1768delC. Patient 24 presented an MTHFR enzyme activity below 7% of control level, while patients 73 and 95 had MTHFR enzyme activities below 1%. All patients presented symptoms of severe central nervous system disease and microcephaly. Two of them suffered a fatal stroke (patient 73 at 18 months and patient 86 at age 14 years). Patient 24 was the mildest, with a diagnosis at age 18 years. Patient 95 could be diagnosed at age 11 months and is improving upon

treatment with betaine. Genotype-phenotype analysis points to less severe outcomes for the patients with missense changes as opposed to the ones whose mutations consisted on gross derangements of the MTHFR protein.

#### P05.135

#### Mutational spectrum of KRT5 and KRT14 genes in patients with epidermolysis bullosa simplex (EBS)

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<sup>1</sup>Department of Genetics, University Medical Centre, Groningen, The Netherlands, <sup>2</sup>Center for Blistering Diseases, Dermatology, University Medical Centre, Groningen, The Netherlands, <sup>3</sup>Centre for Human Genetics, Leuven, Belgium. Epidermolysis bullosa simplex (EBS) is a hereditary skin blistering disorder caused by mutations in the genes for keratin 5 (KRT5) or keratin 14 (KRT14). Mutations disrupt intermediate filament assembly in epidermal basal cells mostly resulting in autosomal dominant EBS. We screened 72 unrelated EBS patients, the majority of which is of Dutch origin. Five patients were from Belgium and one patient was from Slovenia. A total of 20 different heterozygous KRT5 and 15 different heterozygous KRT14 gene mutations were identified in 50 patients. Only one patient carried one missense KRT5 mutation in addition to one missense KRT14 mutation. Each of three mutations, Arg125Cys and Arg388Cys in KRT14 and Arg187Pro in KRT5, were detected in three or more patients. Approximately 80% of the total number of KRT5 and KRT14 mutations were missense mutations. In patients with the severe EBS type Dowling-Meara (EBS-DM) KRT5 mutations were predominantly located at the highly conserved C-terminal 2B domain. Mutations located at the N-terminal H1 and 1A rod domain of the KRT5 protein were detected in patients with the milder EBS type Weber-Cockayne (EBS-WC). In contrast, KRT14 gene mutations located at the N-terminal H1 and 1A domain were mostly present in patients with EBS-DM and KRT14 mutations at the C-terminal 2B domain preferably in patients with the EBS-WC phenotype. We conclude that there is a reverse correlation between mutations located at the N- and C-terminal end of the KRT5 and KRT14 proteins and their subsequent association with either severe Dowling-Meara or milder Weber-Cockayne EBS phenotypes.

#### P05.136

#### A novel family with lamin B1 duplication associated with adult-onset leukoencephalopathy

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Hereditary leukoencephalopathies are rare disorders characterized by progressive white matter damage in the brain, with or without involvement of the peripheral nervous system. The inheritance is usually recessive, autosomal or X-linked. The autosomal dominant varieties include Alexander disease, CADASIL, and the phenotype recently described in four families with adult-onset (40-50 yrs) and initial autonomic dysfunctions due to duplication of the LMNB1 gene (Padiath et al, 2006).

To better define the prevalence of lamin B1 gene defects, we selected eight unrelated Italian probands with hereditary, adult-onset diffuse leukoencephalopathy. Seven showed pyramidal/cerebellar symptoms; six were compatible with an autosomal dominant transmission. LMNB1 full gene deletion/duplication and point mutations were tested by Taq-man real-time PCR and direct sequencing.

One proband carried a 140-190 Kb duplication involving the entire LMNB1, the AX748201 transcript and the 3' end of MARCH3. Clinical and neuroimaging data of this proband and an affected relative overlapped with the features of the LMNB1 duplication described by Padiath et al. No LMNB1 gene defects were identified in the remaining seven probands.

In conclusion, LMNB1 duplication appears to be associated only to a subset of the adult-onset, autosomal dominant leukoencephalopathies, sharing the following features: onset with autonomic dysfunction, diffuse T2-hyperintensity of supra- and infra-tentorial white matter, sparing of U-fibers and optic radiations. The more variable phenotypes in the seven families lacking LMNB1 defects, suggests that other genes might be involved in the autosomal dominant leukoencephalopathies.

#### P05.137

#### Investigation of inflammatory gene expression profile in acute myocardial infarction

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The inflammatory reaction is a key factor influencing the time course of a coronary artery disease and plays a significant role in the clinical outcome of the patients with acute myocardial infarction (MI). Knowing the profile of inflammatory molecules involved in the myocardial injury could contribute to the design of new appropriate therapeutic approaches. Our study aimed to identify the cytokines' type and level produced by the circulating leukocytes by looking for the RNA expression level of the corresponding genes. RNA samples were isolated from blood samples retrieved at different intervals after a MI and analyzed to quantify the expression of 40 proinflammatory genes using the MLPA method.

Our results show an intense overexpression for IL1B, IL1RN, MIF as well as for the NFkB transcription factor, with a peak at 24 h after MI and a slow decrease towards the 30<sup>th</sup> day. Patients associating diabetes mellitus had an excessive inflammatory response with a slower decrease, corresponding to previously reported data. Our study method was able to reliably illustrate the inflammatory profile associated with the MI event. Selected cytokines will be further investigated by RT real-time PCR in order to accurately identify their level and to reveal their contribution to the pathological changes in MI.

#### P05.138

#### The OLR1 splice variant "LOXIN" as a protective genomic biomarker for myocardial infarction.

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The human lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1), encoded by the OLR1 gene, is a scavenger receptor that has been implicated in the pathogenesis of atherosclerosis. LOX-1 activation is an important mechanism that contributes to plaque instability and subsequent development of acute coronary syndromes. Association studies have implicated OLR1 gene variants in myocardial infarction (MI) susceptibility/protection. We identified a new functional splicing isoform of the OLR1 gene, named LOXIN lacking the extracellular lectin binding domain. *In vivo* and *in vitro* studies revealed that this isoform is protective against myocardial infarction. This effect is mediated by a dominant-negative mechanism on LOX-1 function through the formation of non-functional LOX-1/LOXIN hetero-oligomers. Genomic and expression studies performed in mice and in non-human primates demonstrate the absence of LOXIN in these species. This suggests that LOXIN might be a recently selected allele. All these results confirm and extend the importance of OLR1 gene in the pathogenesis of myocardial infarction making LOXIN an attractive new target for prevention and treatment of acute coronary syndromes.

#### P05.139

#### Brain-region specific copper metabolism in rats with copper deficiency induced by Ag-ions

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Copper is an essential trace element that plays a vital role as a catalytic co-factor for a variety of cuproenzymes. Simultaneously, copper

is toxic to cells, not least due to its ability to catalyze, via the so-called Fenton reaction, the generation of aggressive free radicals. Therefore, every organism has mechanisms to control cellular uptake, distribution, detoxification and elimination of copper. Genetic defects in any of these mechanisms as well as ecological abnormalities of copper balance result in neurodegenerative diseases. The systems that maintain copper homeostasis in mammalian brain are investigated poorly. This work was done using Wistar rats received 50 mg AgCl per kg of body weight, daily, in four weeks. This model is characterized with copper-deficit in blood serum and normal copper metabolism in liver. Concentration of copper, zinc, iron, and silver was determined in cortex, cerebellum, hippocamp, hypothalamus, amygdaloid body, hypophysis by atomic-absorptive spectrometry. Copper-transporting proteins (CTR1, ATP7A/B, APP) and cuproenzymes (ceruloplasmin (Cp), GPI-Cp, SOD1, COX (Cox4i1)) mRNA levels were determined with semiquantitative RT-PCR. Protein amounts were valued by Western blot. Furthermore Cp and SOD1 enzymatic activity were measured. It was shown that Ag-ions are selectively accumulated by hypophysis. Hypophysis accumulates Fe and Zn as well. Cu level is decreased in hypothalamus only. At the same time copper-transporting genes expression is going lower in all brain regions. The same is right for secretory cuproenzymes (Cp, GPI-Cp). While level of intracellular cuproenzymes (SOD1, COX) doesn't change. Effect of copper deficit in blood on copper metabolism in brain regions is discussed.

#### P05.140

#### Differential expression of neurokinin B and hemokinin 1 in human immune cells

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Substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and the recently identified hemokinin-1 (HK-1) are members of a family of structurally related peptides known as tachykinins. They are mainly synthesized in the central and peripheral nervous system, but are also present in peripheral non-neuronal cells.

In humans, substance P (SP) is the most extensively studied tachykinin and is present, along with the NK-1 receptor, in several inflammatory and immune cells. In these cells, SP has shown to play an important role in the regulation of immune functions and inflammatory responses. Up to day, there are no studies about the expression pattern of NKB and HK-1 in inflammatory and immune cells in humans. In the present study we have detected for the first time NKB and HK-1 mRNA in human lymphocytes, monocytes, neutrophils and eosinophils. In addition, and using immunocytochemistry with two different antibodies, we have detected the presence of NKB protein in all these cellular types. These findings reinforce the suggestion that tachykinins play a central role in the pathophysiology of the inflammatory process.

#### P05.141

#### Crosstalk between NF-kappaB and Wnt/beta-catenin pathways and anhidrotic ectodermal dysplasia

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Anhidrotic ectodermal dysplasia (EDA) is an ectodermal differentiation disorder characterized by sparse hair, abnormal or missing teeth and inability to sweat. X-linked EDA, caused by mutations in EDA gene encoding ectodysplasin, a member of the TNF family, is the most frequent form. The autosomal dominant and recessive EDA forms, clinically identical to X-linked forms, may result from mutations in two loci : ED3, encoding Edar, ectodysplasin receptor and ED2, encoding Edaradd, Edar adapter molecule necessary in signal transduction. Edar is activated by ectodysplasin and uses Edaradd as an adapter to activate NF-kappaB signaling pathway. Wnt/beta-catenin pathway plays a central role during embryonic development and is widely involved in carcinogenesis. This pathway has been recently involved in skin appendages formation. A crosstalk between NF-kappaB and Wnt/beta-catenin pathway was described.

We confirmed by transactivation experiments that Edar receptor inhib-

its Wnt/beta-catenin pathway. We then studied the effects of seven recessive and dominant mutations identified in Edar gene on both pathways. Dominant mutations totally impaired NF-kappaB activation and Wnt/beta-catenin downregulation, while recessive ones only disrupted the Edar effect on both pathways.

We first demonstrated that Wnt/beta-catenin inhibition by Edar is dependent of NF-kappaB activation using a dominant negative form of IkappaBalpha inhibitor. We then proved by Western Blot analysis and immunofluorescence that beta-catenin was neither degraded nor delocalized after NF-kappaB activation by Edar. Furthermore, beta-catenin/TCF4 interaction, necessary for Wnt/beta-catenin transcriptional activity, was not disrupted upon Edar transfection and NF-kappaB subunits p65 and p50 did not interact with beta-catenin in these conditions.

#### P05.142

#### Nijmegen breakage syndrome in Ukraine: molecular and cytogenetic studies

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Nijmegen breakage syndrome (NBS) is considered as a rare autosomal recessive disease, but its actual prevalence is not known. 95% of NBS patients are of Slavic descent and 90% of them are homozygous for a founder 657del5 mutation in the NBN gene. In 1999-2007 a total of 26 patients were diagnosed with NBS in Ukraine, 23 of which residing in Western Ukraine region.

We performed 208 molecular-genetic tests of the 657del5 mutation NBN gene among the group of patients with microcephaly and identified 24 homozygous for 657del5 mutation. It had been performed 4 prenatal NBS diagnostics and we identified two homozygous and two heterozygous fetus for 657del5 mutation.

We detected the specific chromosomal rearrangements in lymphocyte cell culture of NBS patients: 3% of the cases - inv(7)(p13q35), 2% - t(7;14)(q35;q11), t(7;7)(p13;q35), del(7)(q35), del(14)(q11), 1% - t(7;14)(p13;q11). An additional marker chromosome (10% of the cells with translocations) and nonspecific chromosomal abnormalities (mostly 1, 3, 8 and 9) were found too.

As the expression of IL-10 may influence the development and progression of tumor we studied the distribution of three biallelic polymorphisms at positions -1082, -819 and -592 of the promoter region of IL-10 gene. The haplotype IL-10 GCC (37%) was found to be the most frequent.

#### P05.143

#### Genetic heterogeneity of nonbullous congenital ichthyosiform erythroderma

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Nonbullous congenital ichthyosiform erythroderma (NCIE, MIM 242100) is a severe autosomal recessive disorder. About 90% of affected newborns present as "collodion babies". Many patients die in neonatal period from disorders of thermoregulation and infections. In survivors, though, skin lesions improve with age, few patients have neurological symptoms, while others live with little or no problems. NCIE is genetically heterogeneous. To date, TGM1, ALOX12B, AL-OXE3, ABCA12, CYP4F22 responsible genes were identified, and mutations in the latter two are rare.

We performed DNA diagnostics in five NCIE families of which mutations were found in four: one of Azerbaijan and three of Russian ethnicity. (1) In a consanguineous Azerbaijan family two affected children died within first days, their DNA samples were unavailable. In parents a novel TGM1 mutation Arg142His was detected. On prenatal DNA diagnostics the mutation was not found in the fetus, thus the prognosis was favourable. (2) The couple abandoned the affected baby and had no information about him. Parents were found heterozygous for novel ALOX12B mutations: Ala597Glu and Tyr97Stop. Prenatal DNA diagnostics revealed unfavourable prognosis since the fetus inherited mutations from both parents. (3) In a family with a deceased newborn child a novel ALOX12 mutation Asn594His was identified in the father and a previously recorded Tyr521Cys in the mother. (4) In a

non-consanguineous family the 5-year-old patient was examined. The girl survived infancy problems and by 5 years had only mild ichthyosiform skin lesions. Homozygosity for ALOX12 mutation Ala597Glu was detected.

#### P05.144

#### **Investigation of CYFIP1 and CYFIP2 genes in patients with autosomal recessive non-syndromic mental retardation**

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Autosomal recessive inheritance of non-syndromic mental retardation (ARNSMR) may account for approximately 25 % of all patients with non-syndromic mental retardation (NSMR). Up-to-date, 12 chromosomal loci have been mapped and only four genes have been identified to cause ARNSMR. CYFIP1 (15q11) and CYFIP2 (5q33.3) genes, belonging to CYFIP family by sequence similarity, interests the scientist if any role in mental retardation due to their interactions with FMR1 protein. Suggested function of CYFIP1 in axonal growth and brain specific editing of CYFIP2 was of our further interest to investigate these genes in our highly selected ARNSMR families. 20 consanguineous families with one or more affected individuals were included into our study after through clinical check up to exclude any known syndromes and/or chromosomal or sub-telomeric abnormalities followed by search from fragile-X point of view. Our strategy was to perform homozygosity mapping to CYFIP1 and CYFIP2 genes and sequence the informatively homozygous families. Four in/delSNPs for CYFIP1 gene and four in/del SNPs plus 2 STRs for CYFIP2 gene were selected to be used for linkage. Presently our investigation is continuing and our results will be presented at our poster session.

#### P05.145

#### **NPHS2 recurrent and novel mutations in children from Greece and Cyprus with steroid-resistant nephrotic syndrome**

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The idiopathic nephrotic syndrome occurs mainly in children, most of whom respond well to steroid therapy, however 15-20% of these children are steroid-resistant, frequently progressing to end-stage renal failure. Mutations in the *NPHS2* gene, encoding podocin, are an important cause of infantile sporadic Steroid Resistant Nephrotic Syndrome (SRNS) occurring in 2.8-28% of the cases, depending on the studied population. To a lesser extend, mutations in the *WT1* gene are also found in patients with SRNS. For the first time in Hellenic population, we performed mutational analysis of the *NPHS2* (including promoter region) and *WT1* (exons 8 and 9) genes, studying 18 children from Greece and 6 from Cyprus with SRNS. For our investigation we used a screening method based on the SURVEYOR endonuclease digestion, which identifies and cleaves at mismatched base pairs in heteroduplexes, followed by direct PCR-DNA sequencing. No mutation or polymorphism was identified in exons 8 and 9 of *WT1*. In the *NPHS2* gene, we identified two known mutations (418delG, R229Q) and one novel mutation (L305P), in two out of the 24 children (8.3%), in addition to nine known polymorphisms. Amino acid residue L305 is at the C-terminal of the protein and is highly conserved evolutionarily, possibly because of the functional association of this domain with nephrin and CD2AP. Although the studied cohort was small, our investigation supports the usefulness of the availability of a molecular diagnostic facility for SRNS cases, in a routine clinical setting, as it assists in the correct management of such children.

#### P05.146

#### **LMX1B mutational analysis in two Nail-patella syndrome patients: evidence for the presence of mosaicism**

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Nail-patella syndrome (NPS, MIM#161200) is a rare autosomal dominant disorder characterized by hypoplastic or absent patellae, dystrophic nails, dysplasia of the elbows and iliac horn, and, less frequently, ocular damage.

In the 40% of cases a glomerular defect is involved and inter- and intra-familial expression variability is reported.

Mutations in the human LMX1B gene have been demonstrated to be responsible for NPS in 90% of cases.

In this study we present evidence of somatic mosaicism in two cases of NPS.

The first case is a patient carrying the c.599G>A substitution in exon 4

(Arg200Gln). This mutation was also detectable in the healthy father as a low height A peak superimposed to G. On the contrary, in the patient the two peaks appeared at equal height, as expected in a heterozygote. The second patient is heterozygous for the c.592C>T substitution in exon 4 (Arg198Stop). Also in this case the hypothesis of mosaicism in the healthy father was raised by the presence of the C and T peaks with significantly different height. These analyses were repeated in several independent reactions with fully comparable results. The presence of the mutated alleles in the two fathers was confirmed by subcloning the PCR products: as expected in the hypothesis of mosaicism, the wild-type allele-bearing colonies were significantly more represented than the colonies with the mutated one.

To our knowledge these are the first reported cases of inheritance of a mutated LMX1B allele in NPS patients from a mosaic parent.

#### P05.147

#### **Screening for melanocortin-4 receptor mutations in a cohort of Dutch obese children**

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The most common monogenic form of obesity is caused by mutations in the gene encoding the melanocortin 4 receptor (MC4R). This receptor integrates orexigenic and anorexigenic signals in the hypothalamus to regulate food intake and energy expenditure. We have screened the coding sequence and the minimal promoter region of MC4R of 221 random patients from our VUmc center for childhood obesity. We found 18 variants in these patients, four of which were not described previously. Two of the new variants (-1101C>T and -312T>C) are not expected to be pathogenic because they are not evolutionary conserved in mammals and because they are not located in predicted regulatory regions of the gene. The new variant -705A>T may influence gene expression because it is located in a regulatory element. This variant was found in a 3-year-old girl with a body mass index standard deviation score (BMI-SDS) of 3.4. The new variant 910C>T results in a leucine > phenylalanine change. It was found in a 17-year-old girl with a BMI-SDS of 3.4. Of the 14 known variants that we found, two have been shown to have functional effects by others (Tyr35STOP and G231S). In conclusion, we detected two pathogenic mutations and several possible pathogenic mutations in 221 random patients of our obesity clinic. We will extend our research by screening additional patients, studying cosegregation of mutations and obesity in families, studying phenotypic characteristics of MC4R mutation carriers, and functional studies of new mutations.

**P05.148****Exon resequencing, mutation detection, and copy number analysis in syndromic oesophageal atresia**

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Oesophageal atresia and/or tracheo-oesophageal fistula are common malformations occurring in approximately 1 in 3500 births. In around half of cases (syndromic oesophageal atresia, overlapping with the VACTERL association), there are associated anomalies, cardiac malformations being the most common. Recently, three separate genes with a role in syndromic oesophageal atresia have been identified: those for Feingold syndrome (N-MYC), anophthalmia-oesophageal-genital (AEG) syndrome (SOX2), and CHARGE syndrome (CHD7). It is likely that other genes play a role in foregut development, and dosage sensitive chromosomal loci presumably harbouring these as yet unidentified genes have recently been highlighted (refs 1 and 2).

We are collecting DNA samples from patients with syndromic forms of oesophageal atresia and the VACTERL association. We are analyzing these samples by exon resequencing in the CHD7, N-MYC, SALL1 and SOX2 genes (additional genes, including FANCB and FANCC are currently being added to the panel); and by high resolution array-based comparative genomic hybridization (arrayCGH). To date, we have analyzed 50 samples. No de novo copy number changes have as yet been identified. A de novo frame shift mutation has been detected in CHD7 in a single patient with some features of CHARGE syndrome.

By adopting this comprehensive approach to the analysis of a single malformation, we hope to identify other genes involved in human foregut development and to continue to provide a research-based service to the Clinical Genetics community for this patient group.

1. Shaw-Smith C. *J Med Genet*. 2006; 43(7):545-54.
2. Felix JF, et al *Eur J Med Genet*. 2007; 50(3):163-75

**P05.149****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 syndrome (OFDS). OFDI 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 100 unrelated affected individuals collected worldwide. Putative disease-causing mutations were identified in 81 patients (81%). We describe 67 different mutations, 64 of which represent novel mutations, including 36 frame-shift, 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 exons may represent mutational hotspots. Phenotypic characterization of the patients provided a better definition of the clinical features of OFD type I syndrome. Our results indicate that renal cystic disease is present in 60% of cases with >18 years of age. Genotype-phenotype correlation did not reveal significant associations apart for the high-arched/cleft palate most frequently associated to missense and splice-site mutations. Our results contribute to further expand our knowledge on the molecular basis of OFD type I syndrome.

**P05.150****The Opitz syndrome gene product MID1 assembles a microtubule-associated ribonucleoprotein complex**

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Opitz BBB/G syndrome (OS) is a heterogenous malformation syndrome mainly characterised by hypertelorism and hypospadias. In addition, patients may present with several other defects of the ventral midline such as cleft lip and palate and congenital heart defects. The syndrome-causing gene encodes the X-linked E3 ubiquitin ligase MID1 that mediates ubiquitin-specific modification and degradation of the catalytic subunit of the translation regulator protein phosphatase 2A (PP2A). Here, we show that the MID1 protein also associates with elongation factor 1α (EF-1α) and several other proteins involved in mRNA transport and translation, including RACK1, Annexin A2, Nucleophosmin and proteins of the small ribosomal subunits. Mutant MID1 proteins as found in OS patients lose the ability to interact with EF-1α. The composition of the MID1 protein complex was determined by several independent methods: (1) yeast two-hybrid screening, (2) immunofluorescence, (3) a biochemical approach involving affinity purification of the complex, (4) co-fractionation in a microtubule assembly assay and (5) immunoprecipitation. Moreover, we show that the cytoskeleton-bound MID1/translation factor complex specifically associates with G- and U-rich RNAs and incorporates MID1 mRNA, thus forming a microtubule-associated ribonucleoprotein (RNP) complex. Our data suggest a novel function of the OS gene product in directing translational control to the cytoskeleton. The dysfunction of this mechanism would lead to malfunction of microtubule-associated protein translation and to the development of OS.

**P05.151****Large genomic rearrangements of the OFD1 gene account for 20 % of patients with Oral-Facial-Digital type 1 syndrome and negative DNA sequencing analysis**

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Among the oral-facial-digital syndromes, the oral-facial-digital type 1 syndrome (OFD 1) is characterised by X-linked dominant mode of inheritance. Clinical features include facial dysmorphism with oral, tooth and distal abnormalities, polycystic kidney disease and central nervous system malformations. A high clinical overlap exists between the different types of OFD syndrome. OFD1 DNA sequencing analysis remains negative in 30 to 50% of cases. We hypothesized that large genomic rearrangements could account for the majority of these unexplained cases. A series of 25 index cases (29 patients) with OFD1 phenotype and normal OFD1 DNA sequencing analysis were screened for OFD1 rearrangements by QMPSF and relative quantitative real-time PCR analyses. Five large deletions (exons 1-8, exons 1-14, exons 10-11, exon 17 and exons 13-23) were identified in five index cases, accounting for 20 % of negative mutation patients after DNA sequencing. Among the remaining negative index cases, a family history compatible with dominant X-linked inheritance was found in one case only. Using DNA sequencing, QMPSF and relative quantitative real-time PCR analyses, the OFD1 alteration detection level remains incomplete. However, it is likely that sporadic patients without OFD1 alterations

are affected by another OFD syndrome. Given the percentage of large genomic rearrangements, we suggest that routine molecular screening of *OFT1* gene should include first DNA direct sequencing analysis. QMPSF and relative quantitative real-time PCR analyses could be performed in mutation negative patients, especially in severe cases highly suggestive of OFD1 with a prenatal diagnosis request.

#### P05.152

#### PINK1 mutations and the risk of Parkinson's disease in family members of Southern Italy

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Mutations in PINK1 gene are associated with both familial recessive and sporadic early-onset parkinsonism (EOP). The PINK1 gene codes for a protein (PTEN-induced kinase1) with a catalytic serine/threonine kinase domain. Several groups have demonstrated that PINK1 protein can be localized to mitochondria in vitro. Moreover, functional studies have shown that PINK1 protein may have a neuroprotective role as wild-type PINK1 protects cells against proteasomal inhibition. This protective effect is abrogated by mutations in the PINK1 gene. Herein we investigated a possible association of PINK1 gene mutations in southern Italian families with monogenic parkinsonism.

Fourteen families with PD were investigated for the presence of PINK1 mutations; five had EOP (mean age at onset 36 years) and 9 had familial late-onset disease (LOP, mean age at onset 65 years). Genomic DNA was extracted from blood. PINK1 exons were amplified by PCR and sequenced.

We characterized a novel homozygous mutation (889delG, D297fsX318) in the exon 4 of PINK1 gene in two brothers with EOP from a Sicilian consanguineous family. This mutation causes a frame-shift in translation and a premature termination of PINK1 protein leading to the loss of most of its kinase catalytic domain. No other family members carried the homozygous deletion in the exon 4. We did not find this mutation in 200 chromosomes from healthy Sicilian individuals.

In conclusion, PINK1 mutations are uncommon in our southern Italian families with EOP and LOP. The present study confirms that PINK1 mutations are a cause, even if rare, of EOP in the examined population.

#### P05.153

#### miRNAs in Parkinson Disease

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Parkinson's disease (PD) is the second most prevalent age-associated neurodegenerative disorder, affecting over one million individuals worldwide. The main pathological hallmark of PD is the loss of dopaminergic neurons within the *substantia nigra*, leading to insufficient formation and action of dopamine in the basal ganglia circuitry. The cardinal clinical signs are muscle rigidity, resting tremor, bradykinesia and, in more advanced cases, postural instability.

miRNAs are abundant in the brain and are essential for efficient brain function. Increasing evidence implicates miRNA dysfunction in PD pathogenesis. Although much has been learned in recent years about the genetic aetiology of familial PD, far less is known about the molecular mechanisms underlying the vast majority of cases, which are the focus of our study. To identify novel susceptibility genes for idiopathic PD, we are using the "genomic convergence" approach with microRNA profiling. This strategy converges data from whole-genome linkage studies with expression profiling experiments to determine which candidate genes to test in family-based association studies. We are currently conducting microRNA expression profiles in peripheral blood mononuclear cells of 20 PD patients and 20 controls using microarrays spotted with probes for 452 human microRNAs. The microRNAs differentially expressed and respective target genes mapping to linkage peaks will later be tested for allelic, haplotypic and genotypic associa-

tion with PD. We believe that this approach will allow us to identify specific miRNAs and miRNA target genes playing a role in idiopathic PD.

#### P05.154

#### Mutation screening of exon 3 of *Parkin* gene in a young cohort of Iranian PD patients

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, and the most common movement disorder affecting more than 1% of individuals over 60 years old in Western populations. This is the first genetic study on Iranian PD patients. Mutations in the *Parkin* gene are often observed in patients affected with early onset PD, particularly familial forms of early onset PD. In this study, we screened exon 3 of the gene by direct sequencing in a young cohort of Iranian PD patients. The cohort consisted of 96 patients, with a mean age of onset of 41.8 yrs. (range 10-75 years). The variation c.C245A, which causes substitution of alanine by glutamic acid at residue 82 (A82E) of the coded protein, was observed in three unrelated patients in the heterozygous state. This missense causing variation has previously been reported in control individuals and is unlikely to be associated with disease. One patient seemed to carry a homozygous deletion mutation encompassing exon 3, as this exon failed to be amplified in repeated PCR attempts. Age of onset of PD in the patient carrying the putative deletion mutation was 28 years.

#### P05.155

#### Novel missense mutation in exon 44 of *LRRK2* observed in patients affected with Parkinson's Disease

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Parkinson's Disease (PD) is the second most common neurodegenerative disease. The pathologic characteristics of PD include degeneration of neurons of the substantia nigra in the brain. 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. *LRRK2* is the gene most recently identified and, among identified genes, it seems to be the gene most often mutated in the dominant form of PD. *LRRK2* codes leucine-rich repeat kinase, a huge protein containing 2527 amino acids. The protein has several functional domains, but its role in PD is unknown. We sequenced exons 32 and 44 and flanking regions of *LRRK2* in the DNA of 60 unrelated Iranian PD patients. One variation was observed in exon 32 (c.C4624T causing P1542S) and another in intron 32 (IVS32+347). These variations are thought not to be associated with disease. We also found five variations in exon 44, four of which are intronic and have been previously reported. The fifth variation was G6523C, which was found in the heterozygous state in one patient and causes the substitution of aspartic acid 2175 by histidine (D2175H). This substitution occurs at the N-terminal of the WD40 domain of *LRRK2* which is thought to be a protein-protein interaction domain. The variation was not observed in 218 controls and in 110 additional patients as assessed by ARMS-PCR. Further analysis is needed to confirm the pathogenic effect of this mutation.

**P05.156****Identification of two novel mutations in *LRRK2* among Iranian Parkinson's Disease patients**

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Parkinson's Disease (PD) is one of the most common progressive neurodegenerative disorders. It is characterized by selective loss of nigrostriatal dopaminergic neurons. Five genes associated with PD have been identified by linkage analysis of affected families: *α-synuclein*, *parkin*, *DJ-1*, *PINK-1* and *LRRK2*. Mutations in the *Leucine-rich repeat kinase 2 (LRRK2)* gene have been most often observed in autosomal dominantly inherited PD, a form of the disease which has pathological features most closely resembling those seen in idiopathic forms of PD. Among the approximately 80 exonic variations so far observed in *LRRK2*, less than 10 are definitively thought to cause PD. In this study, we sequenced exons 36 and 37 of *LRRK2* in 60 PD Iranian PD patients. Two novel heterozygous variations, c.G5174A in exon 36 and c.C5467A in exon 37 were observed. Each variation was observed in only one patient. The first causes substitution of arginine by glutamine at position 1725 (R1725Q), and the second causes substitution of glutamine by lysine at position 1823 (Q1823K). Both affected residues are located in the COR (carboxy-terminal of Ras) domain, where at least one pathogenic mutation has been previously identified. To assess the presence of c.G5174A and c.C5467A in other PD patients and in controls, an ARMS-PCR assay was set up for each of the variations. Neither variation was observed among 230 healthy controls and 115 additional unrelated PD patients. Although these observations suggest that both variations may be disease associated mutations, this conclusion is presently not conclusive.

**P05.157****A Yeast Two hybrid screen for LRRK2**

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Mutations in *Lrrk2* cause autosomal dominant Parkinson's Disease. LRRK2 (DARDARIN) belongs to a newly identified family of proteins referred to as ROCO proteins that contain two conserved domains (i) a ROC (Ras in complex proteins) domain that and ii) a COR domain (C-terminal of ROC). In addition LRRK2 also contains a WD40 and a leucine rich repeat domain, as well as a predicted tyrosine kinase catalytic domain. A yeast two hybrid was performed to help elucidate the function of LRRK2.

LRRK2 was found to interact with fasciculation and elongation factor zeta 2 (FEZ2), a mammalian orthologue of the *Caenorhabditis elegans* UNC-76 protein, which is involved in the axonal outgrowth and synaptic organization. FEZ2 is ubiquitously expressed in mammalian tissues with the majority of information about its function being inferred from studies of its homolog, FEZ1. FEZ1 appears to be involved in neurogenesis upon phosphorylation by PKCζ. Knockout of UNC-76 from C-elegans leads to locomotion and axonal transport defects. Given the role of FEZ1 in axonal outgrowth and normal synaptic function, FEZ2 and LRRK2 may co-operate in maintaining SN neuritic length and branching, and makes FEZ2 a good functional interactor for LRRK2. In support of this hypothesis, recent evidence has suggested that LRRK2 plays a role in maintaining neuronal morphology in vitro and in vivo.

**P05.158****Effect of overexpression of PKD2 in a murine model**

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Polycystin-2 (PC-2), a cation channel of the Trp family, is involved in autosomal dominant polycystic kidney disease (ADPKD) type 2 (AD-

PKD2). This protein has recently been localized to the primary cilium where its channel function seems to be involved in a mechanosensory phenomenon. However, its biological function is not totally understood, especially in tubule formation. We describe a mouse model for human PC-2 overexpression, obtained by inserting a human BAC containing the PKD2 gene. Three lines were generated, expressing different levels of PKD2. One line, PKD2-Y, has been explored in more details and we will present physiological and molecular exploration of this transgenic animals. Our data demonstrate that transgenic animals older than 12 months present tubulopathy with proteinuria and failure to concentrate urine. The kidney cortex has been found disorganized. Moreover, we observe that extracellular matrix protein expression is downregulated in these animals. Overexpression of human PKD2 leads to anomalies in tubular function, probably due to abnormalities in tubule organogenesis. Surprisingly, PKD2-Y FISH analysis showed that a significant number of metaphases presented with more than a single Y chromosome. Transgenic nucleus size is larger compared to non-transgenic cells suggesting problems during cell division. Likewise we show that fibroblasts from transgenic mouse present with mitotic instability. It remains to be determined what is the relationship between PC-2 and cell division.

**P05.159****Gene expression in 2-3 somite stage WT and Pkd2 mutant mouse embryos**

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The development of an invariant left-right (LR) asymmetry of the visceral organs is a fundamental feature of vertebrate embryogenesis. Failure to establish the normal organ position (*situs solitus*) may result in a mirror-image reversal (*situs inversus*), in left or right isomerism or in heterotaxia (*situs ambiguus*). While the complete *situs inversus* does not have adverse impact on the organisms, heterotaxia frequently is accompanied by fatal malformations and complex cardiac and cardiovascular defects. Increasing evidence based on research on genetically modified animals including our *Pkd2* knockout mouse suggest, that the disturbance of proper LR axis development and thus misalignment of the developing heart tube is one of the major causes for the development of congenital heart disease (CHD) resulting in early prenatal death in most cases and it is very likely that the same mechanisms are responsible in large part for early abortions in the first trimester of pregnancy in humans.

To identify and to characterize novel genes and mechanisms which influence LR axis development in vertebrates and thus might be involved in the development of congenital cardiac defects we performed a gene expression analysis of right and left body halves of 2-3 somite stage male WT and *Pkd2* mutant mouse embryos using Affymetrix Gene Chips. We identified and validated by *in situ* hybridization (ISH) analyses several genes which are either already known or highly suggestive to influence LR axis development. Data obtained during this analysis will be presented.

**P05.160****Genetic mechanisms involved in the pathogenesis of premature ovarian failure: study of *FMR1*, cryptic anomalies (especially small deletions) in the Xq27-Xqter region and other causative genes, in 122 women from the Basque Country.**

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Premature Ovarian Failure (POF) is characterized by secondary amenorrhea, elevated level gonadotropins and sex steroid deficiency in women less than 40 years old. It is well known that some of the Premutation (PM) carrier women of Fragile X Syndrome (FXS) develop POF. More recently, it has been identified a common region responsible for ovarian development -Xq27-Xqter-, where the *FMR1* gene lies and interestingly, so lie other X-linked genes for MR. Furthermore, there are other genes in the pathogenesis of POF, the results being that it is heritable in up to 30% of cases.

We studied all of these genetic mechanisms in peripheral blood sam-

ples of three groups of women: 42 known PM females from families of FXS, 25 with POF and 17 without; and 80 females with POF, and normal FMR1. The following genes were analysed: FMR1; BMP15; GDF9; INHA; NOBOX and FOXE1 polyalanine tract length. Two kits of MLPA for the X chromosome were also studied.

Our results revealed 3 new PM carrier women without history of MR (3/80=3.75%) which confirms that FMR1 gene is the first cause of POF. Furthermore, we found a microdeletion by MLPA (Xq28-Xqter) (1/80=1.25%) in a woman with POF and a family history of POF. In the group of previously known PM carrier women no differences were found except for the number of CGG repeats that is higher than 100 in the group without POF.

As a conclusion, our work demonstrates the need of performing genetic analysis in women with POF.

#### P05.161

#### Acute intermittent porphyria: impact of mutations in the porphobilinogen deaminase gene on the protein structure

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Acute intermittent porphyria (AIP) is an autosomal dominantly inherited disorder, classified as acute hepatic porphyria. It is characterized by a deficiency of porphobilinogen deaminase (PBGD, EC 4.3.1.8), the third enzyme in heme biosynthesis. Expression of the disease is highly variable, determined in part by environmental, metabolic and hormonal factors. Clinical expression of AIP is associated with an acute neurologic syndrome, which includes both neuropsychiatric symptoms and neurodegenerative changes. Autonomic neuropathy may underlie severe abdominal pain, the most typical symptom of acute attack. Porphobilinogen deaminase is monomeric protein with a single catalytic active site and is organized in three domains approximately equal in size. Several crystallographic structures of PBGD from *E. coli* have been determined.

To establish the effects of newly found mutations on the protein structure of PBGD, we expressed mutant constructs with the described mutations in *E. coli*, and we analyzed their biochemical and enzymatic properties. All purified enzymes carrying causative mutations had relative activities dramatically decreased according to the average level expressed by the normal allele.

*E. coli* and human PBGD amino acid sequences have 35% homology and more than 70 % similarity, and considering this fact, it is possible to extrapolate structure/function relationships for human mutations leading to simple amino acid substitution based on comparative *E. coli*/human analyses. These analyses together with the kinetic studies of existing mutations can help predict the impact on the enzyme function in the living organism and further improve our understanding in this field. (Supported by Grants MSM0021620806, 1M6837805002, GAUK 257540 54007)

#### P05.162

#### Molecular characterization of Premature Ovarian Failure associated with FMR1 premutation

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Premature Ovarian Failure (POF) is defined as secondary hypergonadotropic (FSH ≥ 40 IU/l) amenorrhea occurring before the age of 40, and affects ~1% of females. Women who carry the FMR1 premutation are at an increased risk for ovarian insufficiency. To precisely define the range of FRAXA expansions and its incidence in POF manifestation 209 POF and 200 control women were sized for the CGG tract. We found a significant association (10%, P < 1x10-6) between POF and FRAXA premutation (range 60-163 repeats), and a significant enrichment (5.7%, P = 0.006) of POF carriers of intermediate expansions (range 41-58 repeats). The results obtained strengthen the correlation between FMR1 expansion and POF. The molecular pathogenesis this association is still unclear. As for FXTAS patients, a toxic RNA-mediated gain of function model for POF women carrying the FMR1 premutated allele was hypothesized. The POF condition might be a consequence of a toxic effect of FMR1 expanded mRNA

on ovarian target tissue (e.g. oocytes or granulosa cells), leading to increased atresia/apoptosis of follicles. To determine the possible interaction between premutated FMR1 RNA and ovarian proteins, gel shift assay were set up incubating the nuclear extracts of the granulosa cell line COV434 with premutated FMR1 RNA. A specific interaction between granulosa nuclear proteins and FMR1 premutated RNA were observed, suggesting that rCGG-repeated binding protein are present in granulosa cells. To identify and characterize rCGG-repeat-binding proteins, pull-down assays will be set up.

#### P05.163

#### Novel type of mutation in Pseudohypoparathyroidism type Ia

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Individuals with AHO and resistance to PTH, TSH, and often, additional hormones are referred to as having PHP-Ia. These patients carry heterozygous inactivating mutations, including translation initiation mutations, amino acid substitutions, nonsense mutations, splice site mutations and small insertions or deletions, in one of the 13 GNAS exons encoding the α-subunit of the stimulatory G-protein (Gsa). GNAS has been described as one of the most complex gene loci as several different transcript variants have been detected.

Objective: We sought to identify the molecular defect in a patient apparently suffering from PHP-Ia.

Methods and results: The GNAS gene of a 5-yr-old boy with brachydactily, mental retardation, pseudohypoparathyroidism, and congenital hypothyroidism was investigated. Sequencing analysis of DNA extracted from peripheral blood revealed a heterozygous mutation in exon 2, consisting in an inversion of de novo origin and maternal inheritance, that was explained by the analysis of the SNP located in exon 5.

Molecular studies of cDNA prepared from blood RNA demonstrated that both the normal and the mutant variants were stable. The anomalous RNAs were generated by new splice-sites in intron 1, that produced a protein of only 59 aminoacids due to the creation of a premature stop codon in the processed intron 1.

Conclusion: This report demonstrates for the first time an inversion at the GNAS gene responsible of pseudohypoparathyroidism type Ia.

#### P05.164

#### Deficient inhibitors of calcification act as common final pathway in PXE and the PXE-like syndrome

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Pseudoxanthoma elasticum (PXE) is an autosomal recessive disorder, characterized by oculocutaneous and cardiovascular manifestations, due to mineralization and fragmentation of elastic fibres. The causal ABCC6 gene encodes a transmembrane transporter; however, the pathogenetic link with the elastic fiber abnormalities remains unknown.

We recently identified a novel PXE-like syndrome - resembling PXE -, associated with a vitamin K (VK)-dependent clotting factor deficiency. We identified causal mutations in GGCX, encoding a gamma-carboxylase. Together with phosphorylation by the Golgi casein kinase, carboxylation represents an important posttranslational modification necessary for activation of VK-dependent proteins, such as matrix gla protein (MGP) and osteocalcin (OC), known to be calcification inhibitors. Hence, this disorder provides novel possibilities to unravel the pathogenetic events causing PXE.

In PXE-like patients, immunohistochemistry on skin tissues and serum ELISA tests revealed increased levels of uncarboxylated (inactive) MGP and OC, as a result of the GGCX mutations. In PXE patients, similar results were obtained. An important difference in PXE patients was however an increase of the phosphorylated fraction of inactive MGP, whereas the phosphorylated/unphosphorylated MGP ratio in PXE-like samples was normal. This suggests that in PXE not only the carboxylase activity is downregulated but also an upregulation of the

Golgi casein kinase occurs.

We demonstrated that dysfunction of (VK-dependent) calcification inhibitors forms a common pathway in the PXE-like syndrome and PXE. Our findings suggest that ABCC6 dysfunction does not merely lead to decreased serum levels of its substrate but causes a chain of intracellular events involving carboxylation and phosphorylation of calcification inhibitors.

#### P05.165

##### Characterization of a novel mutation in the CTSK Gene in a family with PYCD

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Pycnodynatosis is an inborn error of metabolism due to the deficient activity of cathepsin K, a lysosomal cysteine protease from the papain family of proteases. The autosomal recessive disorder is consequence of the diminished capacity of osteoclasts to degrade organic bone matrix. Pycnodynatosis is clinically characterized by short stature, osteosclerosis, delayed cranial suture closure, hypoplastic mandible, acro-osteolysis, hypoplastic clavicle and dental anomalies. The spine may be affected with kyphosis, scoliosis or lumbar lordosis. Pathological fractures, nonunion of fractures and spondylolisthesis are frequent complications in pycnodynatosis. The gene of the pycnodynatosis (CTSK) has been mapped on the human chromosome 1q21. A few CTSK gene mutations have been identified in several non-related families with pycnodynatosis. In the present study, we analyzed the CTSK gene in three members of a family with pycnodynatosis and identified a novel missense mutation. The parents agreed to participate. Whole blood was obtained from each patient as well as from the parents and 100 normal controls. Genomic DNA was extracted from whole blood samples. All exons of the CTSK gene were amplified and sequenced from genomic DNA of the patients, parents and controls by PCR and DNA sequencing analysis. We found a novel mutation in the carboxyl extreme of the cathepsin K. This mutation, and previous data, show that affection of carboxyl-terminus of the enzyme is important in the genesis of pycnodynatosis phenotype.

#### P05.166

##### Copy Number Variations and Long-QT Syndrome

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Long-QT Syndrome (LQTS) is an inherited cardiac arrhythmia characterized by a prolonged QT interval on the surface ECG associated with syncope and sudden death caused by torsades de pointes polymorphic ventricular tachycardia. It is estimated to affect 1 in 5000 individuals. LQTS may be caused by mutations in 4 major genes encoding potassium channel pore forming (KCNQ1, KCNH2) and auxiliary (KCNE1, KCNE2) subunits and in the gene encoding the cardiac sodium channel SCN5A. Approximately 25% of patients diagnosed with LQTS have no mutation in one of five LQTS genes. Genetic testing detection failures due to large genomic rearrangements are one explanation. The purpose of this study was to determine the relative copy number in the 5 major LQTS genes in 100 mutation-negative LQTS probands. A MLPA approach was used and aberrant exon copy numbers were confirmed using Agilent 244K CGH array.

This study identified 2 large deletions in KCNH2 gene in 2 probands with QTc intervals of 478 ms and 554 ms. The first patient carries an estimated 145 Kb deletion including KCNH2, and ABP1 genes. The second deletion spanning 650 Kb includes KCNH2 exon 4 to 15, ABP1 and 18 additional genes. Familial investigations identified 3 additional affected individuals carrying the KCNH2 deletion. Both deletions are expected to be non functional, decreasing I<sub>Kr</sub> current in ventricular cardiomyocytes, suggesting haploinsufficiency as the most likely mechanism leading to LQTS.

The identification of 2% large deletions in LQTS genes strongly suggests screening for copy number variants in mutation negative LQTS probands.

#### P05.167

##### Mitochondrial DNA mutations and recurrent miscarriage

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The mitochondria are involved in ATP production and apoptosis. These processes are important in early development and may be disturbed by mutations in mtDNA, thereby leading to spontaneous miscarriage. Phenotype causing mutations are usually heteroplasmic and cause a phenotype when the proportion of mutant mtDNA increases beyond a threshold. This proportion can shift at cell division/when inherited and therefore the phenotype often shows remarkable variation within a family. Accordingly, such a shift can occur from a mother with mild or no phenotype to an embryo with significant enrichment of mutated mtDNA causing fetal demise and possibly recurrent miscarriage (RM).

To study the role of mtDNA mutations in RM we screened 48 women with RM for mitochondrial mutations using dHPLC, a sensitive method which can detect ~5% heteroplasmy. So far, 10 different heteroplasmic variations have been detected. By screening placental samples available from 3 women with different variations we have been able to show that the variations have been inherited by the fetus, and are in some cases present in a higher proportion of mtDNAs in the fetus compared to the mother. So far, we have been able to determine the exact sequence change of three variations. Two changes are previously reported in the Mitomap database as normal variations. One variation is not reported in the database and is predicted to be a synonymous change in the mtND6 gene. Further studies are ongoing to define the remaining variations to determine if the variations may be the actual cause of miscarriages.

#### P05.168

##### Validating the role of RET in the CNS: discovery of novel interactors

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RET encodes a tyrosine kinase receptor mainly expressed in neural crest derived and urogenital cells. Two major isoforms of RET are produced by alternative splicing: RET51 and RET9.

Mutations of RET protooncogene have been associated with both neoplasia (MEN2A, MEN2B and FMTC) and Hirschsprung disease.

The precise role of RET in the maturation of the peripheral nervous system, in kidney morphogenesis and in spermatogenesis has been established. RET is responsive to signals induced by neurotrophic factors of the GDNF-family ligands.

Recent studies have shown that RET binds the neurotrophin NGF. This suggests a functional role of RET in the Central Nervous System (CNS). Nonetheless, no signaling pathway involving RET is known in the CNS. Hence we developed a strategy aimed at identifying novel interactors that may help us to reconstruct the pathway connecting RET and NGF. To accomplish this task, we applied to RET51 the technique of the yeast two hybrid split ubiquitin system, that allows detection of interacting molecules belonging to the cytoplasm and the membrane. A first screening for RET 51 interactors has revealed 10 candidates. Among these we focused on a neurotrophic factor. Validation of the interaction was carried out through a co-immunoprecipitation assay in HEK293 cells. We either ascertained that this interaction occurs via a specific amino acid residue activated after binding of NGF.

On the basis of these preliminary results, we are currently testing the hypothesis that RET 51 might have a central role in the growth and the trophism of CNS cells.

#### P05.169

##### A high-resolution RNA expression atlas of Retinitis Pigmentosa genes in the human and mouse retinas

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Retinitis Pigmentosa (RP) is one of the leading causes of visual handicap in the world population and is characterized by high genetic heterogeneity. The study of the disease mechanisms and the development of efficient therapeutic approaches have mostly relied on the availability of animal models for this condition, so far. Nevertheless, little information is available about the RNA expression profiles of RP genes in the human retina. To overcome this lack of information, we generated an expression atlas of 34 known RP genes in human and murine retinas. The atlas can be freely accessed at <http://www.tigem.it/RPexp/>. The vast majority of the genes analyzed displayed similar patterns between human and mouse retina. We observed different expression patterns for the CNGB1, USH2A and FSCN2 genes with respect to previously reported profiles. Additionally, we detected different expression profiles for the RPGR, CA4, PAP1, RGR and RLBP1 genes in human and mouse retina. The differences observed in the expression patterns of some genes in human and mouse will open new perspectives on the function of these genes and on their putative roles in disease pathogenesis.

#### P05.170

##### **Overexpression of CERKL, a Retinitis Pigmentosa gene, protects cells from apoptosis under oxidative stress conditions**

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Retinitis Pigmentosa (RP) is a highly heterogeneous genetic disease, where more than 30 causative genes have been already reported. RP is characterised by progressive retinal neurodegeneration due to photoreceptor apoptosis. Our group identified a previously unannotated gene, CERKL, as responsible for this disease in 3 different Spanish families. CERKL encodes a presumptive lipid kinase that shares similarity to the human ceramide kinase. Ceramides are sphingolipids, a group of membrane lipids that have been increasingly involved in the regulation of relevant cellular and physiological processes, such as cell growth, differentiation, apoptosis and inflammation. Therefore, we hypothesised that CERKL would play a key role in controlling photoreceptor survival/death. Overexpression of the CERKL enzyme in transiently transfected cultured cells clearly protects cells from apoptosis under oxidative stress conditions. This protection starts as early as 4 hours, but becomes more prominent at 24 hours post-treatment, where apoptosis is reduced two-fold by CERKL expression. We are now actively searching for its retinal substrate. Our in vitro and in vivo preliminary results do not support that ceramide/s are the direct substrate for this enzyme. Subcellular localisation of CERKL shows that it is associated to membranes, such as endoplasmic reticulum, Golgi and Trans-Golgi vesicles, although it can also be found in the nucleus in some cells, pointing to shifts in localisation depending on the cellular state. Dissecting the function of CERKL will provide new clues on the sphingolipid role on photoreceptors and unveil new targets for therapeutic approaches.

#### P05.171

##### **Mutational analysis of RHO and RDS genes in patients with autosomal dominant form of retinitis pigmentosa from Volga-Ural region of Russia**

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Purpose: To identify mutations in the RHO and RDS genes in patients with autosomal dominant form of retinitis pigmentosa (adRP) from Volga-Ural region of Russian Federation. Retinitis pigmentosa, the most common hereditary cause of blindness, comprises a clinically and genetically heterogeneous group of retinal disorders. It affects about one in 5 000 individuals worldwide

Methods: 37 patients with adRP confirmed by pedigrees were examined clinically and with visual function tests. The 5 exons of RHO and 3 exons of RDS genes were analyzed for sequence changes by single-strand conformation polymorphism analysis (SSCP) and further sequencing.

Results: We detected known mutation Pro347Leu, novel mutation Arg-252Pro and two polymorphisms IVS1+10g>a, IVS3+4c>t in RHO gene.

There were statistically significant differences in allele and genotype frequencies of sequence change IVS3+4c>t of RHO gene in affected patients with adRP and in controls. According to our data, this polymorphism might be pathogenic. Recently were reported 16 possible combinations of the exon 3 RDS gene four SNPs and detected four from 16 possible combinations (minihaplotypes): G<sup>1147</sup>A<sup>1166</sup>G<sup>1250</sup>C<sup>1291</sup> (I), C<sup>1147</sup>A<sup>1166</sup>A<sup>1250</sup>C<sup>1291</sup> (II), C<sup>1147</sup>G<sup>1166</sup>A<sup>1250</sup>C<sup>1291</sup> (III) and G<sup>1147</sup>A<sup>1166</sup>G<sup>1250</sup>T<sup>1291</sup> (IV). Sequencing results for variant positions in exon 3 RDS gene in adRP patients from Volga-Ural region revealed all four minihaplotypes described above and minihaplotype C<sup>1147</sup>A<sup>1166</sup>A<sup>1250</sup>T<sup>1291</sup> (V). Minihaplotype C<sup>1147</sup>A<sup>1166</sup>A<sup>1250</sup>C<sup>1291</sup> (II) was revealed only in patients and it might be linked with RP.

Conclusions: To optimize the DNA diagnostics of retinitis pigmentosa, it is necessary to analyze patients from various ethnic groups. Our study helps in molecular characterization of RP in Russia.

#### P05.172

##### **Knock-down of Cox5a in HEK293 cells**

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Cox5a is one of 13 structural subunits of cytochrome c oxidase (COX), the terminal enzyme of respiratory chain. We used RNA interference (RNAi) to down-regulate steady-state level of Cox5a subunit and analyzed impact of its knock-down on COX assembly.

We constructed seven derivatives of pCMV-GIN-ZEO plasmid coding for hairpins aimed at different positions of COX5A mRNA. A Cox5a protein has a long half-life (a level of the protein is 48 hours post-nucleofection unchanged using immunoblotting). Therefore we introduced COX5A coding sequence into the maxFP-Red - N plasmid to encode fusion protein. The marker plasmid was co-transfected with RNAi-mediating plasmid derivatives (the higher the level of fusion protein the higher the leakage of individual hairpin-mediated RISC systems). A fluorescence of fusion protein was found rapidly lower compared to maxFPRed marker thereby complicating a setting of FACS measurements, but Western Blot of fusion protein with COX5A antibody gave an acceptable result. To optimize the detection of RNAi-potential through fluorescence by FACS, we re-cloned COX5A coding sequence into 3'UTR of maxFPRed marker. The final transcript contains target sequence for RNAi but leads to translation of merely maxFPRed marker. Fluorescence intensities were comparable with that obtained at empty plasmid.

Based on the above-mentioned methods, we chose three candidate shRNAs and prepared stable cell lines, where depletion of Cox5a was confirmed using SDS immunoblotting. Also specific activity of COX was revealed decreased. BN-PAGE showed diminished level of COX holoenzyme and its assembly intermediates.

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#### P05.173

##### **The challenges encountered in diagnostic screening of the RYR1 gene**

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Mutations in the skeletal muscle ryanodine receptor gene (*RYR1*) are implicated in a number of congenital myopathy phenotypes including central core disease, multi-minicore disease and centronuclear myopathy.

We have developed a high throughput 384-well sequencing approach for screening the 106 exons of *RYR1*. This method uses tagged primers and robotics to overcome the practical challenges faced by screening such a large gene. To date we have tested over 44 patients and here we report on the complexities which have arisen.

Certain *RYR1* related disorders may exhibit both autosomal dominant and recessive modes of inheritance; it is not always certain whether we are searching for one or two mutations. The identification of missense changes of unknown pathological significance complicates this.

Our mutation screening is further complicated due to the marked phenotypic variability: mutations in *RYR1* can manifest in a wide range of clinical phenotypes whilst similar phenotypes may result from mutations in a number of different genes excluding *RYR1*. Additionally there are reports of *RYR1* mutations that only exhibit symptoms on a mono-allelic background.

One particularly interesting case involves a patient with a clinical diagnosis of autosomal recessive multi-minicore disease in whom we found three RYR1 mutations - two missense mutations previously reported as pathogenic and one previously unreported three base pair deletion.

In addition to causing congenital myopathy, mutations in certain regions of the RYR1 gene are also associated with malignant hyperthermia susceptibility (MHS) and it is important that the implications of this are clearly explained to other family members.

#### P05.174

#### Role of ATTCT repeat interruptions in spinocerebellar ataxia type 10

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Spinocerebellar ataxia type 10 (SCA10) is a dominant neurological disease, caused by the expansion of an (ATTCT)n in intron 9 of ATXN10, of still unknown function. SCA10 was first described in Mexican families with ataxia and seizures. We have previously described an expansion of the ATTCT repeat in two Brazilian families presenting ataxia without seizures. We found reduced penetrance alleles of 360-370 repeats, in elderly asymptomatic subjects.

To investigate a previous hypothesis of interruptions in the (ATTCT)n tract, functioning as a disease modifier, we assessed the interruption in another family with ataxia and seizures, and more than 2000 ATTCT units.

By a modified PCR technique, an abnormal discontinuous ladder, exceeding the range observed for normal alleles, was detected in this Brazilian family, presenting progressive cerebellar ataxia with associated seizures and onset during or after the 3<sup>rd</sup> decade. This suggested the presence of interruptions within the ATTCT expansion. Modified PCR products of patients showing an interrupted pattern were agarose-gel purified and cloned with TOPO TA cloning kit for sequencing analysis and determination of the interrupted motif.

In this family, the ATTCT repeat seems to be interrupted by a large stretch, unrelated to the repeat sequence, and patients show progressive cerebellar ataxia with associated seizures. In contrast, the previous SCA10 families identified by us, showed cerebellar ataxia without seizures, caused by the expansion of uninterrupted ATTCT tracts. This newly identified family reinforces the hypothesis that interruptions in the (ATTCT)n tract function as a disease modifier.

#### P05.175

#### Ribosomal frameshifting on expanded ATXN3 transcripts: a Drosophila model

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**Background:** Spinocerebellar ataxia type 3 (SCA3) is caused by the expansion of a coding CAG repeat in the ATXN3 gene. We have previously shown that the expanded CAG repeat in SCA3 is prone to -1 ribosomal frameshifting, leading to the production and aggregation of proteins containing polyalanine stretches. These frameshifted molecules confer increased toxicity to cells when compared to constructs containing expanded CAA repeats, which code for polyglutamine in the main frame but lack the ability to frameshift into an alanine frame. Anisomycin (a ribosome interacting antibiotic that reduces -1 frameshifting) decreases frameshifting in expanded CAG tracts and ameliorates the cellular toxic phenotype.

**Aims:** To model expCAG repeat -1 frameshifting *in vivo*; to assess the contribution of -1 frameshifting to expCAG toxicity in the fly.

**Methods:** Full-length ATXN3 *Drosophila* transgenic lines carrying either wtCAG, expCAG or expCAA constructs containing epitope tags in the three possible reading frames were generated and comparatively analysed.

**Results:** We show that: (1) transgenic expression of expCAG ATXN3 constructs is deleterious in the fly; (2) transgenic expression of exp-

CAA ATXN3 constructs, despite adequate levels of protein expression, is not toxic; (3) -1 frameshifting occurs in *Drosophila* and is restricted to the expanded CAG transgenic lines.

**Conclusions:** We propose that -1 ribosomal frameshifting is a major contributor to the toxicity observed in expanded CAG repeat diseases. This novel pathological mechanism may open new therapeutic opportunities for these diseases.

#### P05.176

#### Mutation analysis of the SCN1A gene and genotype-phenotype correlations in Bulgarian epilepsy patients

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Epilepsy is a common neurological disease, affecting more than 1% of people from any age, gender and ethnical origin. Mutations in the alpha1-subunit ( $\alpha 1$ ) of the neuronal voltage gated sodium channel  $\text{Na}_v 1.1$ , are associated with generalized epilepsy with febrile seizures plus (GEFS+) and severe myoclonic epilepsy in infancy (SMEI). It is encoded by 26 exons of the SCN1A gene on chromosome 2q24.

We performed a mutation analysis of SCN1A in 28 patients with GEFS+, 20 with SMEI and 62 with other types of infantile epilepsy. Genomic DNA was extracted from peripheral blood lymphocytes using a standard sodium chloride precipitation method. The SCN1A point mutation screening included PCR analysis followed by direct sequencing of all exons and exon-intron boundaries of the gene. In addition, all patients were analyzed for exonic deletions and duplications by MAQ assay.

We identified 11 disease-causing mutations, including 6 missense, 2 nonsense, 1 splice-site mutation and 2 single base deletions. Two missense and all truncating mutations were found in SMEI patients, consistent with the severity of the epilepsy phenotype. Four GEFS+ patients carried a missense mutation. All mutations we identified had not been reported in the literature. This is the first large scale study of SCN1A gene analysis in Bulgarian epilepsy patients.

In our sample, a mutation was identified in 23% of patients with GEFS+ or SMEI, and none in the other epilepsy patients. This nicely illustrates that GEFS+ and SMEI are part of a continuous spectrum of fever associated epilepsy phenotypes caused by mutations in SCN1A.

#### P05.177

#### KPQ del in SCN5a gene in two different types of SCD in one family

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Cardiac action potentials are generated and propagated through the coordinated activity of multiple ion channels, including voltage-gated sodium channels, calcium channels and potassium channels. Mutations in genes encoding these channels cause familial arrhythmias.

Heart rhythm disturbances constituent some inherited syndromes any kind of genetic nature

Many kinds of arrhythmia caused SCD (sudden cardiac death) and the prevalence of SCD is 1: 1000 individuals. Some of SCDs were caused by Na channelopathy such as LQT3 and Brugada Syn these are two of main caused of sudden cardiac death in young people with out any detectable cardiac abnormality with routine tests and we know that Brugada syndrome was caused by loss of function of Na channel and LQT3 was caused by gain of function Na channel in heart.. We can check SCN5a gene for ruling out of this gene as a main gene of Na channelopathy in heart arrhythmias. We check by SSCP and Sequencing this gene. We checked SCN5A in a family with 11 affected person that approved by ECG and EPS study and some persons

had Brugada Syn and some had LQT3 .we checked SCN5a and we can find KPQ del 1505-1507 in all patients.As you know this del was caused only LQT3.so it was very interesting that we can find this del in Brugada syndrome. So we are working in other hypothesis for solving this situation

#### P05.178

#### Seckel-syndrome like phenotype associated with a 550 kb microdeletion of the long arm of chromosome 5 characterized by array comparative genome hybridization (aCGH)

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Primordial dwarfism and Seckel-syndrome are rare genetically heterogeneous disorders. Several subtypes of these severe growth retardation syndromes have been described mainly based on clinical features, growth characteristics, presence or absence of particular skeletal anomalies and further criteria. During the last years a remarkable progress in identification of genes involved was achieved. Seckel-syndrome and primordial dwarfism can be genetically caused following an autosomal recessive mode of inheritance. Genes involved in cell division, cell cycle control pathways or related to DNA repair have recently been identified and characterized to be responsible for some forms of these disorders when a homozygous loss of function mutation is present. Here we report on a 3 ½ year old male patient resembling phenotype features of Seckel-syndrome associated with a 550 kb microdeletion 5q33 detected by high resolution aCGH. The patient is the fifth child of young healthy non-consanguineous parents. Birth weight was 800 g, length 37 cm. No precise information about gestational week at birth was available. Weight at 3 ½ years is 4.450 g, length 62.4 cm and head circumference 39.8 cm. He shows a beaked nose, low set ears, micro- and retrognathia, transverse palmar creases, fifth finger clinodactyly, general hirsutism and darker skin pigmentation than relatives. Psychomotor development is good, however he is unable to speak because he has a tracheostoma since more than two years. Investigation of those less than 20 genes deleted and the molecular rearrangement could allow identifying a gene responsible for a variant form of primordial dwarfism or Seckel syndrome.

#### P05.179

#### Severe Combined Immunodeficiency (SCID) caused by R235Q homozygous mutation in adenosine deaminase (ADA) gene

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Inherited deficiency of ADA accounts for approximately 17% of SCID and 50% of autosomal recessive SCID.

Patient: a two months old boy from Morocco with a suspicion of SCID (severe infections, diarrhea, failure to thrive, profound lymphopenia). Parents were consanguineous (first cousins). Erythrocyte ADA enzyme activity was assayed: ADA activity in patient was nearly undetectable while in his parents it was about 50% of a control. In his healthy brother activity was normal.

Molecular diagnosis: nearly 70 different mutations have been described in ADA gene causing SCID; half of them are "private". Most patients are heteroallellic and homozygosity is mostly restricted to consanguineous families.

Mutational analysis of ADA gene was performed. We designed primers for PCR amplification of the 12 exons comprising the gene. PCR products were purified and sequenced with the amplification primers using BigDye Terminator Cycle Sequencing kit in an ABI 310 Genetic Analyzer.

Results: The patient was homozygous for R235Q (704G>A) missense mutation in exon 8. Both parents were heterozygous for this mutation. His healthy brother was also heterozygous, in spite of his normal ADA activity. It is the first time that R235Q mutation is detected in homozygosity in a SCID patient, and the first time this mutation is detected outside of Japan.

Patient was also homozygous for D8N (22G>A) polymorphism in exon 1; parents and brother were carriers. This polymorphism is not related to SCID but it causes decreased enzyme activity and can contribute to the low level of ADA activity in the patient.

#### P05.180

#### Mutations in SHOX2 do not appear to be involved in Léri-Weill dyschondrosteosis (LWD) and idiopathic short stature (ISS)

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SHOX encodes a transcription factor involved in determining stature in humans. Mutations/deletions of SHOX and a downstream region result in Léri-Weill dyschondrosteosis (LWD), Langer mesomelic dysplasia and idiopathic short stature (ISS). However, no molecular defect has been detected in a variable proportion of LWD/ISS cases. SHOX has a human homolog, SHOX2, located on chromosome 3q25.32, which function is unknown. The Shox2-/- mouse presents a cleft palate phenotype with shortened limbs, suggesting that SHOX2 may participate in limb development.

In order to investigate if SHOX2 is implicated in LWD/ISS, we have analyzed 35 LWD/ISS families and 89 ISS individuals, without a known molecular defect. SHOX2 mutation screening was performed by DH-PLC and DNA sequencing of the exons and intron/exon boundaries. Large deletions were analyzed in the familial cases using previously described (D3S3692) and novel microsatellites (D3S4638, D3S4639 and D3S4640) flanking SHOX2.

Two variants were identified in exon 1 of SHOX2 in two familial cases: a missense mutation E21K and a duplication of three glycines Gly77-Gly78dup (c.232\_233dupGAGGAGGTG). We subsequently screened exon 1 in 50 healthy individuals. The E21K mutation was not observed in controls but it did not cosegregate with the short stature phenotype. Insertions and deletions of the glycine repeat in SHOX have been described in patients with LWD/ISS but the insertion in the glycine repeat of SHOX2 appears to be polymorphic as we observed duplications of this region in three normal controls.

These results suggest that SHOX2 is not the molecular cause of the LWD/ISS in the cases studied.

#### P05.181

#### Pathways of SOD1 and TAU-induced motor neuron degeneration

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Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease that selectively affects motor neurons. To identify early molecular triggers of selective motor neuron degeneration, we applied microarray technology to investigate gene expression profiles of motor neurons and glia isolated with laser capture microdissection from two distinct transgenic mouse models of ALS: SOD1-G93A and TAU-P301L. The analysis was performed prior to the development of neurodegeneration reducing the number of false positives due to the possible reactive changes in the course of the disease. The majority of identified genes are model specific, indicating that TAU and SOD1 induced neurodegeneration have distinct molecular pathways and therefore, may require specific treatments. Several genes were consistently altered in the motor neurons of both models. One of them, Dynein, points to the early dysfunction of retrograde axonal transport. To investigate the relevance of microarray identified gene expression changes for human sporadic disease, immunohistochemical experiments were carried out using a specific ALS tissue microarray (TMA) consisting of ALS, FTD and control human postmortem CNS tissues.

#### P05.182

#### Alu-related 5q35 microdeletions in Sotos syndrome

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Haploinsufficiency of the NSD1 gene due to 5q35 microdeletions or intragenic mutations causes Sotos syndrome (SoS). In 46 of the 49 Japanese deletion-cases, common deletion breakpoints were located at two flanking low copy repeats (LCRs), implying that non-allelic homologous recombination (NAHR) between LCRs is the major mechanism for the common deletion. In the remaining three cases of atypical

deletions, the mechanism(s) of deletions remains unanswered. We characterized the atypical microdeletions using fluorescence in situ hybridization (FISH), quantitative real-time PCR (qPCR), and Southern blot hybridization. All the deletion breakpoints in the three cases were successfully determined at the nucleotide level. Two deletions are 1.07 Mb (SoS19) and 1.23 Mb (SoS109) in size, and another consisted of two deletions with sizes of 28 kb and 0.72 Mb, intervened by an intact 29-kb segment (SoS44). All deletions were smaller than a typical 1.9-Mb common deletion. *Alu* elements were identified in both deletion breakpoints in SoS19, one of deletion breakpoints in SoS109, and both deletion breakpoints of the proximal deletion in SoS44. *Alu*-mediated NAHR is strongly suggested at least in two of atypical deletions. Finally, qPCR is a very useful method to determine deletion breakpoints even in repeat-related regions.

#### P05.183

##### Sotos syndrome. Mutational study in 186 patients

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Introduction. Sotos syndrome (SS) is a rare genetic condition characterized by overgrowth, macrocephaly, large forehead, large hands and feet, accelerated bone age, typical gestalt, mental retardation of variable degree and a slight predisposition to develop tumours. This condition is due to an abnormality of the *NSD1* gene caused by deletions or spontaneous mutations.

Material and methods. From November 2003 to November 2007, we evaluated 215 patients with presumed diagnosis of SS, referred to the Spanish Overgrowth Syndrome Registry. The patients were evaluated in their local hospitals and referred by geneticists, paediatricians, neurologists and other health professionals. An initial study by microsatellite genotyping, a MLPA specific for the *NSD1* critical region and neighbouring genes and analysis of the coding regions of *NSD1* by dHPLC and/or bi-directional direct sequencing were made in all of them.

Results. We completed all the studies in 186 out of the 215 patients referred. One hundred and twelve presented complete clinical data or a clear phenotype of SS, evaluated by 2 clinical geneticists trained in SS patients. We found 76 genetic alterations, 17 of them *NSD1* deletions and 59 point mutations; a detection rate of about 68%. Some of these mutations are novel and others are recurrent.

Comments. The detection rate of mutations in patients with clear clinical-neurobehavioural phenotype of SS is nearly 70%. Our figures are similar to that reported in other series. The finding of several recurrent mutations suggests that there are "hot spots" in *NSD1*, meaning common molecular mechanisms responsible for the disease.

#### P05.184

##### Identification of the *SPG15* (Spastizin) gene, responsible for an autosomal recessive complicated spastic paraplegia

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Hereditary spastic paraplegias (HSPs) are genetically and phenotypically heterogeneous disorders with various clinical profiles and modes of transmissions. The *SPG15* locus was first reported to account for a rare form of complicated autosomal recessive spastic paraplegia variably associated with mental impairment, pigmented maculopathy, dysarthria, cerebellar signs, distal amyotrophy and thinning of the *corpus callosum*, sometimes designated Kjellin syndrome. Here, we report the refinement of the *SPG15* locus to less than 3 Mb on chromosome

14q23-q24 and the identification of 6 different truncating mutations that were found to segregate with the disease in 8 families with a phenotype that included, variably, some of the clinical features of Kjellin syndrome. The *SPG15* mRNA was widely distributed in human tissues and in rat embryos as well. In adult rodent brain, its expression profile closely resembled that of *SPG11*, another gene responsible for complicated HSP. The identification of the *SPG15* gene is a starting point for the elucidation of the mechanisms underlying axonal degeneration in this complicated form of HSP.

#### P05.185

##### Mutation spectrum and phenotype description in a large series of patients with autosomal recessive spastic paraplegia type 11 (SPG11)

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Hereditary spastic paraplegias (HSP) are neurodegenerative disorders mainly characterized by lower limb spasticity associated, in complicated forms, with additional neurological signs. Recently, we identified the *SPG11*, as the responsible gene for a complex autosomal recessive (AR) HSP. We have analyzed 76 index ARHSP patients for mutations in the *SPG11* gene. We found 22 mutations segregating in 13 (30%) families and 7 (21%) isolated cases. Two mutations were recurrent suggesting founder effects. Nineteen mutations were novel. The highest frequency of patients with *SPG11* mutations (41%) was found in HSP patients presenting with a thin *corpus callosum* (TCC); however, these mutations were rare in patients with mental impairment without TCC (4.5%). Disease onset occurred during the first to the third decade mainly by problems with gait and/or mental retardation. Overall, the phenotype of the 38 examined *SPG11* patients was severe. After a mean disease duration of  $14.9 \pm 6.6$  years (range: 2-35), 53% of patients used a wheelchair or were bedridden. At the time of examination, in addition to mental retardation, 80% of the patients showed cognitive decline with executive dysfunction. The phenotype also frequently included lower motor neuron degeneration (81%) with wasting (53%). Slight ocular cerebellar signs were also noted in patients with long disease durations. In addition to TCC (95%), brain MRI revealed white matter alterations (69%) and cortical atrophy (81%), which worsened with disease duration. Our study reveals the high frequency of *SPG11* mutations in patients with HSP associating TCC and cognitive impairment and also extends the associated phenotype.

#### P05.186

##### SPG4 mutations and phenotype of hereditary spastic paraplegia in Estonia

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Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous disorder, which is classified clinically into "pure" (pHSP) and "complex" (cHSP) forms. Great inter- and intrafamilial variations are very typical. Autosomal dominant hereditary spastic paraplegia (AD-HSP) is the most common form due to the mutations in the spastin gene (*SPG4*). The aim of this study was to describe phenotype

and to detect new sequence variants within SPG4 gene in 51 HSP patients in Estonia. Fifty healthy individuals were used as controls. The majority of patients had pHSP and only five patients had cHSP form of the disease. cHSP was the most frequent in sporadic cases. All 51 samples were screened with DHPLC and abnormal elution profiles were sequenced. Nine changes (G609A, A810G, 1299-1g>c, 1310delA, 1370+215g>c, 1370+202delG, C1503A, 1477-1481del-GAGAA, 1966insA) in SPG4 gene were detected showing no gender predisposition. Seven were new and only found in 11 HSP patients, but two (one new - 1370+215g>c and other previously described - 1370+202delG) were detected both in patients and controls. Only two mutations (1299-1g>c, C1503A) showed familial segregation, in which three and two family members were affected, respectively. Other mutations were found in individuals from different families. In conclusion we associate above-mentioned seven new mutations with 11 AD-HSP cases, since new sequence variants were found only in patients compared to healthy controls. The rest of the patients must be checked for other changes (larger INDELs) in SPG4 and other genes involved in this disease should be considered for further analysis.

#### P05.187

#### In depth investigation of -1 frameshifting in expanded CAG repeat tracts using time-lapse live cell imaging

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Spinocerebellar ataxia type 3 (SCA3) results from an expansion of a polyglutamine-encoding CAG tract in the ATXN3 gene. We have previously demonstrated that this expanded CAG tract is subject to -1 ribosomal frameshifting into the alanine frame, which seems to confer an increased toxicity, and that the antibiotic anisomycin reduces both -1 frameshifting and cell toxicity. Currently, dual-tagged ATXN3 reporter constructs were created to express DsRED in the main (glutamine) frame and EGFP in the -1 (alanine) reading frame, and these reporter constructs contained either 14 CAG repeats, 89 CAG repeats, or 92 CAA repeats. Constructs were transfected into COS-1 cells as well as mouse cortical organotypic slice culture preparations, and were monitored for the production of red or green fluorescence signals using time-lapse live-cell two-wave fluorescent microscopy. Employing this technique, we have confirmed the occurrence of -1 frameshifting for the CAG89 construct, whereas the constructs bearing wild-type CAG or expanded CAA repeats did not show significant frameshifting. We also determined that there is a marked time delay between the onset of glutamine-containing protein expression and the production of frame-shifted species, as well as a correlation between frameshifting and cell death. These findings argue in favour of local glutamine codon starvation, followed by a shift in the reading frame to resume translation of the protein in the alanine frame and seem to confirm the implication of -1 ribosomal frameshifting in the pathogenesis of SCA3.

#### P05.188

#### Quantitative analysis of SMN1 gene based on real-time PCR

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder, caused by the homozygous absence of the survival motor neuron gene (*SMN1*) in approximately 94% of patients. Since most carriers have only one *SMN1* gene copy, several quantitative analyses have been used for the SMA carrier detection. We performed a *SMN1* quantitative real-time PCR analysis using an allele specific primer for the carrier detection of SMA. We compared the sensitivity, specificity, advantages and disadvantages recently described in the quantitative method, using TaqMan probes and a newly developed Plexor™ technology, which had not previously been used for identifying heterozygotes. Using a comparative threshold cycle ( $C_t$ ) method and the DNA fragment of human beta globine gene as a reference gene, the gene copy number of *SMN1* was quantified. The sensitivity and specificity of the TaqMan and Plexor™ technologies were similar; moreover, the assay efficiency was almost ideal when using the Plexor™ technology. The incidence of SMA (1:6000-10000) and the notable carrier

frequency (1:35-50) as well as the severity of disease, and the lack of effective treatment may justify the implementation of such analysis in DNA diagnostic laboratories.

#### P05.189

#### Molecular Genetics and Epidemiology in Spanish Spinocerebellar Ataxia

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Autosomal dominant cerebellar ataxias (ADCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders in which several spinocerebellar ataxia (SCA) genes have been cloned: SCAs1-3, SCAs6-7, SCA12 and SCA17; sharing a CAG repeat expansion mutations which generally encodes a polyglutamine tract. In SCA8 the mutation is an untranslated CTG repeat. We have analyzed 340 unrelated familial and 1,013 sporadic and idiopathic cases of SCA. Over the familial cases 6.04% were SCA1; 26.85% SCA2; 32.89% SCA3; 7.38% SCA6; 6.71% SCA7; 14.77% SCA8; and 1.34% SCA17. In 22 familial index cases with SCA8 expansions the allele range goes from 85 to 470 repeats (129.36% ± 67.55%; Pearson Coef. = 52.22%). Maternal transmissions presented elongations of the CTG combined sequence ranging from +2 to +13 repeats (7.50 ± 5.5; Pearson Coef. = 73.33%). In contrast, paternal transmissions presented contractions ranging from 1 to -17 repeats (-6.83 ± 7.51; Pearson Coef. = -109.93%). Several giant SCA8 expansions ranges from 401 to 1,126 (N= 9), carried by unaffected adult individuals and being originated from homozygous SCA8 females with alleles of moderate size. In contrast, the homozygous males have transmitted contracted alleles, as in heterozygous cases occurs. We have tested 90 individuals from general population and the distribution of SCA8 alleles could be classified in two groups: 15 to 34 CTGs, with frequency 98% and 77 to 86 CTGs, with frequency 2%. About 60% of familial ADCA cases remained genetically unclassified. No SCA mutations were detected in the 1,013 isolated and idiopathic cases of spinocerebellar ataxia.

#### P05.190

#### Novel mutations found in ABCA4 in Spanish families

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Introduction: ABCA4 mutations have been associated with Stargardt disease (STGD). A few cases with autosomic recessive cone-rod dystrophy (arCRD) and autosomic 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.

Subjects and Methods. 31 unrelated families, were previously studied with the ABCR400 genotyping microarray. In patients with either none or only one mutant allele were analysed by dHPLC, sequencing and multiplex ligation-dependent probe amplification (MLPA). Haplotype analysis was also performed.

Results. 27 ABCA4 mutations were found in 31 Spanish patients with the ABCR400 microarray. We confirmed that the p.Arg1129Leu mutation is the most frequent in Spanish patients. dHPLC allowed us to find eleven novel mutations and were not found in the 100 chromosomes. Using both tools, the mutation detection rate obtained was incremented in a 24.2% with respect to the use of the microarray alone. We detected 1.6 % of false positives and 1.6 % of false negatives. In 17/31 patients (54.8%) in which the second or neither mutation was found by these methodologies, they were studied with MLPA; however no deletion or duplication was found in these samples. No mutation was found in 4/31 patients (12.9%).

Conclusions. The ABCR400 microarray is a comprehensive screening tool for genetic variation in patients with ABCR-associated retinal pa-

thology. We consider that the combination of microarray, dHPLC and MLPA is the optimal screening strategy for mutation analysis in this huge gene in Spanish patients.

#### P05.191

#### Genomic Structural Variation in patients with Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease that affects the central nervous system. Several studies provide evidence that MS is a complex disorder involving genetic and environmental factors. Several different approaches have been undertaken to elucidate the genetic causes of MS, including linkage and association studies. These have consistently shown association to the major histocompatibility complex (MHC) region, specifically the DR15 haplotype. Other MS genes have been more elusive, and only ILR7 has shown up as a clear risk factor. The aim of this study was to evaluate a possible contribution of genomic structural variation to MS susceptibility. Forty relapsing-remitting MS samples were divided into two pools and comparative genomic hybridization (CGH) against a pool of 50 control samples was performed using Agilent 244K arrays. With the initial selection criteria (three consecutive probes with a log<sub>2</sub> ratio above 0.29), only the chromosome 6 MHC region, spanning four to six probes (depending on the pool), showed differential hybridization between cases and controls. Relaxing the criteria to only two consecutive probes, five additional regions showed a difference in cases versus controls and were selected for follow-up by RTqPCR experiments. In addition, based on the association shown by the MHC region, a SNplex experiment with 48 SNPs from HLA class II region was designed, including SNPs from potential CNV regions and SNPs not analyzed by other platforms, and three of these SNPs were shown to be significantly associated to MS.

#### P05.192

#### Analysis of raw data from MLPA-based assays: development of a new web-based software „eMLPA“

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MLPA allows detection of large deletions/duplications that would otherwise remain undetected by standard PCR-based techniques. MLPA is based on relative quantification of PCR-multiplexed specific sequences, and for interpretation, computerized analysis of data is necessary. Available commercial, proprietary or freely distributed software packages differ in user friendliness, diagnostic utility, sensitivity and specificity. Usually, these software applications also do not allow optimization of utilized algorithms by long-term "feedback learning" from stored data.

Our "eMLPA" software aims to provide a universal computational interface for all MLPA-based assays. While other software packages use defined algorithms for data analysis, eMLPA allows selection of variant methods for e.g. probe normalization. As none of the individual steps (peak discrimination, e.g.) is a trivial task, instead of rigid computations our approach suggests solutions to be verified, corrected or rejected in interaction with users. Data transformation via independent procedures allows for flexibility and comparison of results at various analytical steps. Data manipulation (management of users, probes, samples and results) is implemented in a web-based system, with no other requirements than a browser. To support the logistics of teamwork properly, eMLPA allows users to create, manage and collaborate on defined projects. eMLPA also offers long-term storage of anonymized intermediary data for evaluation of different numerical methods, for data quality control or for assessment of variance of results of specific probes within a given MLPA mix.

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#### P05.193

#### Gene expression signature of adrenaline biosynthesis and inflammatory pathways in women with left ventricular apical ballooning syndrome

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Marked elevation of plasma catecholamine levels has been reported in transient left ventricular apical ballooning syndrome (LVABS).

Using quantitative PCR (Q-PCR), we investigated the expression profiles of inflammatory and adrenergic pathways in the RNA isolated from peripheral leukocytes, and from lymphocyte and monocyte subsets of 16 female patients with LVABS, and compared with 7 age-matched women with acute myocardial infarction (AMI) and 17 presenting with chest pain and angiographically normal coronary arteries. Three of the 351 genes belonging to the adrenaline biosynthesis pathway [DAT (Neurotransmitter transporter, dopamine), AT (Sodium-dependant amino acid transporter) and NET (Norepinephrine transporter)] were over-expressed in LVABS than in AMI, whereas COMT (Catechol-O-methyltransferase) and PMNT (Phenylethanolamine N-methyltransferase) were under-expressed. Similarly 3 of the 343 genes of the inflammatory pathway [IL1 $\beta$  (Interleukin 1 $\beta$ ), TNF $\alpha$  (Tumor necrosis factor-alpha), IL10 (Interleukin-10)] were overexpressed in AMI while TBX21 (T-cell-specific T-box transcription factor) in LVABS; although higher in LVABS, MAD-homolog was underexpressed in both AMI and LVABS than in controls; CCR5 (Chemokine receptor 5) was underexpressed in AMI than in LVABS and controls. In addition 3 of 178 genes of the angiogenesis pathway [AT1R (Angiotensin receptor 1), AT2R (Angiotensin receptor 2) and HIF2A (Endothelial-pas domain protein 1)] were overexpressed in the AMI than in LVABS and controls, while iNOS was more expressed in controls than LVABS and AMI. The lymphocytes did not express DAT, NET, AT, DOPA and COMT.

Our gene expression results suggest an overactivity of adrenergic and inflammatory pathways in LVABS and replicate the biochemical profile documented previously in LVABS.

#### P05.194

#### Research Report: Analysis of the TCR transcriptome by TcLandscape: a tool for T cell immune response characterization and patient follow-up

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The understanding of T cells during the immune response follow-up is a crucial step in the development and improvement of immunotherapy treatments. TcLand has developed a new workflow and tool: the TcLandscape. This method provides a global and detailed analysis of the T Cell Receptor (TCR)  $\beta$ -chain transcriptome. The TcLandscape consists of a quantitative analysis of TCR  $\beta$ -chain mRNA expression by real-time PCR, followed by a qualitative diversity study and T cell selection based on the collection of the TCR Complementarity Determining Region 3 (CDR3) length distribution (CDR3-LD) with a capillary electrophoresis sequencer. An integrated analysis is then performed to assess the contribution of each V $\beta$  chain length with the total T cell repertoire. The result is displayed in a 4-dimensional visualization graph. Novel dedicated statistical analysis methods, including specific signal treatment algorithms and multidimensional data reduction methods, allow the identification of significant differences between the different groups of patients.

The ability to analyze whole T cell populations, as well as specific T cells, such as CD4+/CD8+ or Treg cells, provides a powerful tool to highlight sub-populations of interest in order to understand auto-immune diseases, transplantation, vaccination and other immune responses. In this presentation, we will outline the workflow to conduct a TcLandscape study.

#### P05.195

#### Characterization of a spontaneous, recessive, missense mutation arising in the *Tecta* gene

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The *TECTA* gene encodes alpha-tectorin (*TECTA*), a major non-collagenous component of the tectorial membrane (TM). In humans, mutations in *TECTA* lead to either dominant (DFNA8/A12) or recessive (DFNB21) forms of non-syndromic hearing loss. All missense mutations in *TECTA* that have been reported thus far are associated with the dominant subtype, whereas those leading to recessive deafness are all inactivating mutations. Here we characterize a spontaneous missense mutation (c.1046C>A, p.A349D) arising in the mouse *Tecta* gene that is, unlike all previously reported missense mutations in *TECTA*, recessive. The morphological phenotype of the *Tecta*<sup>A349D/A349D</sup> mouse resembles, but is not identical to, that previously described for the *Tecta*<sup>ΔENT/ΔENT</sup> mouse. As in the *Tecta*<sup>ΔENT/ΔENT</sup> mouse, the TM is completely detached from the surface of the organ of Corti and spiral limbus, lacks striated-sheet matrix, and is deficient in both beta-tectorin (*Tectb*) and otogelin. A significant amount of *Tecta* is, however, detected in the TM of the *Tecta*<sup>A349D/A349D</sup> mouse and numerous, electron-dense matrix granules are seen interspersed amongst the disorganized collagen fibrils. Mutated *Tecta*<sup>A349D</sup> is therefore incorporated into the TM but presumably unable to interact with either *Tectb* or otogelin. The *Tecta*<sup>A349D/A349D</sup> mouse therefore reveals that missense mutations in *Tecta* can be recessive and lead to TM detachment, and suggest that should similar mutations arise in the human population, they would likely cause deafness.

#### P05.196

#### Molecular genetic analysis of the Wiskott-Aldrich syndrome in four Russian families

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The Wiskott-Aldrich syndrome is a rare X-linked recessive immunodeficiency disorder caused by mutations in the *WASP* gene.

*WASP* gene located at Xp11.22-p11.23, has 12 exons containing 1823 base pairs.

A search for *WASP* gene mutations was performed by direct DNA sequencing analysis of all exons and exon-intron junctions.

Four different *WASP* gene mutations were found in the four unrelated families with Wiskott-Aldrich syndrome.

In the first case only biological material of an affected proband's mother was available for searching for mutations. The frameshift mutation (p.Phe36fs44) was found in the exon 1 of *WASP* gene in heterozygosity. Also, the prenatal diagnosis of Wiskott-Aldrich syndrome was carried out for this family. In the second case the missense mutation (p.Asp224Gly) was found in the exon 7. Proband's mother was heterozygous for the mutation. In the third and fourth families the splice site mutations were found in the intron 8 of *WASP* gene - IVS 8 + 1nt G → C and IVS 8 + 1nt G → A, respectively. Probands mothers were heterozygous for found mutations. Also, in the last case proband's brother had the same mutation and maternal aunt and grandmother were non carriers of this mutation.

One novel mutation p.Asp224Gly was found in this molecular investigation, the others have been reported. We supposed the mutations "hot spot" in intron 8 of *WASP* gene.

#### P05.197

#### Hereditary forms of thrombophilia in patients with fetal loss in the Kazakh population

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The aim of this research is to detect hereditary forms of thrombophilia in patients with syndrome of loss of fetus in the Kazakh population.

We investigated the genotype frequencies of the C677T mutation of the MTHFR gene, the G20210A mutation of the prothrombin gene and the Factor V Leiden mutation in 100 Kazakh patients with a history of fetal loss and 200 healthy female as controls. The hereditary thrombophilias were found in 53% of patients. The C677T mutation was present in 41% of patients, 35% women were heterozygous and 6% patients were homozygous. This mutation was found in isolation in 28% of patients, and in combination with other mutations in 13%. The C677T mutation was found in 22% of controls, from them 21%

are heterozygotes and 1,5% were homozygous. The Leiden mutation was found in 9% of patients and all were heterozygotes. In 4% of patients the Leiden mutation was the only abnormality. It was present in combination with other mutations in 5%. In contrast, the factor V Leiden mutation was found in only 1,3% of controls. The G20210A mutation of the prothrombin gene was found in 4% of patients, and all are heterozygotes. The mutant was the only abnormality in 2% of patients and it was present in combination with other mutations in a further 2%. In controls this mutation was not found. The combination of two and even of three defects of thrombophilia were found in 18% of patients whereas in the control group we found only isolated defects of thrombostasis.

#### P05.198

#### Screening for the C46T polymorphism in the F12 gene by melting point analysis with the Light Cycler system: atypical results, detection of the variant G47A

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The C46T in the F12 gene is a novel genetic risk factors for thromboembolic disease. Thus we designed a method, based on melting peak analysis using fluorescence hybridization probes (Real Time PCR-Lightcycler, Roche), to genotype the C46T as a routine analysis. We report the results of one case that showed atypical melting curves in the LightCycler analysis.

The patient was a 53-year-old man with cerebral ischemia diagnosed by computed tomography. A genetic analysis showed that he was heterozygote for the prothrombin G20210A mutation and showed atypical melting curves when the C46T polymorphism was analyzed. The family study revealed that his father, his sisters and one son were also carriers of the G20210A and they showed the same atypical melting curves for C46T. Subsequent sequencing revealed a G47A heterozygous variant. This variant was not detected in 250 healthy controls indicating that this variant is extremely rare.

Although the G47A variant is rare, its position adjacent to the C46T polymorphism imposes some diagnostic limitations in that C46T might be undetectable. In addition individuals with C46T exhibit low levels of FXII because this variant generates an alternative initial methionine and as consequence a stop codon resulting in the synthesis of a premature protein. The presence of both, G47A and C46T in such close positions prevents the generation of an alternative methionine and consequently cancels the effect of C46T.

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#### P05.199

#### A transporter of the thyroid hormone T3 (MCT8) is implicated in X-linked dysmyelination (Pelizaeus-Merzbacher Like Disease / Spastic Paraplegia Type 2)

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Among the numerous genes implicated in X-linked mental retardation, the one coding for the specific thyroid hormone T3 transporter, i.e. monocarboxylate transporter 8 (MCT8, SLC16A2, MIM 300095), has been initially implicated in boys presenting a severe delay of psychomotor development and secondly in a syndromic mental retardation affection characterized by a progressive spasticity, the Allan-Herndon-Dudley syndrome (MIM 300523). In those clinical pictures, abnormal relative concentrations of thyroid hormones (total T4 decreased, TSH borderline and total T3 increased) suggest the implication of this gene. The identification of a MCT8 missense mutation in one of our patients presenting a diffuse hypomyelination on MRI imaging until the age of 3 years old has inclined us to test the implication of this gene in X-linked early dysmyelination suggestive of Pelizaeus-Merzbacher Like disease or spastic paraplegia type 2. MCT8 coding sequences have

been analyzed by DHPLC in a cohort of 37 boys presenting a hypomyelination without mutation in the PLP1 gene. A MCT8 mutation has been identified in 4 patients from 4 independent families. These results demonstrate the interest of MCT8 mutation screening in hypomyelinating leukodystrophies of unknown origin and suggest, at least in humans, a new role of MCT8 in oligodendrocyte maturation.

#### P05.200

##### Alopecia areata: Association with resistance to thyroid hormones

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**Context :** Resistance to thyroid hormone (RTH) syndrome is a rare genetic disorder, generally characterized by the absence of the usual symptoms and metabolic consequences of thyroid hormone excess. Mostly it is caused by thyroid hormone  $\beta$  receptor mutations. Up to date 124 mutations in the TR $\beta$  gene identified in 500 different families. Besides, alopecia areata (AA) is an autoimmune disease of the hair follicle, frequently associated with other autoimmune disorders, one of the most common of which are thyroid diseases like hashimoto thyroiditis and Graves' disease.

**Objective:** we described a family having RTH syndrome, caused by a novel TR $\beta$  mutation, coexisted with diffuse, patchy alopecia without autoimmune thyroid disease in affected members

**Design:** For the precise diagnosis of RTH, genetic testing was carried out on the index patient, two siblings, his mother and father. Genomic DNAs were extracted from peripheral blood samples by using standard protocols. Exon 7-10 of the thyroid receptor beta (TR $\beta$ ) were amplified by PCR and possible candidate mutations screened by using direct DNA sequencing.

**Main Outcome:** A novel TR $\beta$  mutation (I353V) was found in a 9 1/3 years old boy having both RTH syndrome and alopecia areata. This mutation was also detected in his father and elder brother who were affected by RTH and also have alopecia areata. It could be Speculated that alopecia areata might be a novel sign of RTH either coexisted with the other distinctive clinical and phenotypical characteristics of the syndrome.

#### P05.201

##### Functional characterisation of single nucleotide polymorphisms in the coding region of the human tryptophan hydroxylase gene TPH2

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The tryptophan hydroxylase isoenzyme 2 (TPH2) catalyzes the rate-limiting step of serotonin synthesis in the human brain. Reduced serotonin synthesis may be linked with neuropsychiatric disorders like major depression or bipolar disorder or with the success or failure in therapy of these diseases. Several genetic variants in the coding region of the TPH2 gene have been reported but, thus far, only few of them have been functionally characterized. Here we present functional analysis of the TPH2 variants L36Q, P206S, P312P, A328V and D479E.

The enzyme variants were heterologously expressed in PC12 and HEK293 cells. Enzyme activity of the protein variants was assessed as amount of intracellular serotonin or 5-hydroxy-tryptophan measured by HPLC. The expression rates were controlled by qRT-PCR and Western blot.

The 328V variant showed significantly decreased activity both in PC-12 (only 28% of the wild type activity,  $P<0.002$  Mann-Whitney U test) and HEK293 cells (16%,  $P<0.05$ ). The P206S substitution led to decrease of the TPH2 activity in HEK293 cells (32%,  $p<0.05$ ), but not in PC-12 cells. The L36Q and D479E substitutions showed no effect on the enzyme activity in both cells tested. The common silent variant P312P did not effect enzyme expression or activity. Furthermore, no effect of the P312P variant on the TPH2 splicing could be shown in an exon-trapping assay.

In conclusion, the A328V substitution leads to significant reduction of the TPH2 activity *in vitro*. The role of this variant in the susceptibility or in the therapy of serotonin-related neuropsychiatric disorders reminds to be elucidated.

#### P05.202

##### Analysis of the TSC1 and TSC2 genes in patients with Tuberous Sclerosis from Spain

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Tuberous Sclerosis is a multisystemic disorder characterised by hamartomas frequently found in brain, skin and kidney. Convulsions, seizures and mental retardation are also common features of the entity. TS is caused by mutations in TSC1 (9q34) and TSC2 (16p13) genes. Both are tumour suppressor genes and encode the hamartin (TSC1) and tuberin (TSC2) proteins. We report here our findings after the TSC1/TSC2 genetic study of patients coming from several hospital Spanish services. Mutational analysis were performed by DNA sequencing. Our results are discussed in basis of the relation between clinical data of patients and the gene mutation variant associated. We have familial and sporadic cases and in both we also try to establish the number of minimum criteria for candidate selection to genetic testing.

#### P05.203

##### What about UMOD gene involvement in renal development? A novel UMOD mutation associated with immature renal structures

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Mutations of the UMOD gene, encoding uromodulin, have been associated to medullary cystic kidney disease (MCKD), familial juvenile hyperuricemic nephropathy (FJHN) and glomerulocystic kidney disease (GCKD).

We report on an 13-year-old boy, presenting with moderate chronic renal failure, a family history (father) of an undefined renal-transplantation-requiring nephropathy and a personal history of hyperuricemia and urine concentrating ability impairment preceding the onset of renal failure. Renal ultrasonography demonstrated slightly reduced bilateral kidney volumes and cortical hyper-echogenicity, with two tiny cysts in the left kidney. Renal biopsy showed up to 60% of glomeruli featuring an enlargement of Bowman's space (glomerular cysts), with mild interstitial fibrosis (αSMA-positive), inflammatory infiltrate and focal tubular atrophy at the cortical level. At the cortico-medullary junction, immature tubules (some dilated), with PAX2-positive immunostaining, surrounded by a vimentin-positive mesenchymal tissue. Unlike previously reported cases, no uromodulin positive globular aggregates within the cytoplasm of tubular cells were observed.

Genetic analysis revealed a novel heterozygous mutation of UMOD gene (c.149 G>C;p.Cys50Ser), involving the first EGF-like domain of the protein, both in the boy and his father.

This novel UMOD mutation, associated with an immunohistochemical pattern different from the previous reports and a histological picture characterized by immature renal structures, opens up new issues about UMOD possible role in renal development.

#### P05.204

##### PDZK7 does not participate in the usher protein network

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**Introduction:** Usher syndrome (USH) is an autosomal recessive genetic disease defined by the association of sensorineural hearing loss and visual impairment due to retinitis pigmentosa (RP), with or without vestibular affection. USH is clinically and genetically heterogeneous. Nine genes have been identified which codify for proteins that are integrated in a protein network known as Usher interactome. The central core of the interactome is formed by the PDZ domain containing homologues harmonin and whirlin with the rest of USH proteins assembling

to this core. Additional interacting proteins assembling to the USH protein network are candidates to be responsible for Usher syndrome, non-syndromic hearing loss or retinal dystrophies.

**Hypothesis:** The protein product predicted for PDZK7 (NM\_024895) shows high homology to both harmonin and whirlin and could have a similar function. Thus, it is a candidate member for the Usher interactome.

**Objective:** The aim of the present work was to determine the existence of interactions between PDZK7 and the proteins of the Usher interactome: usherin, NBC3, VLGR1, cadherin 23, protocadherin 15 and SANS.

**Methods:** Interactions were tested by yeast two hybrid (Y2H) assays and by co-expression and co-localization in cos-1 cells studies.

**Results:** No interaction was found between PDZK7 and USH proteins.

**Conclusions:** The absence of interactions between PDZK7 and USH proteins indicates that it is not part of the USH interactome.

#### P05.205

#### Assessment of hearing loss in French patients diagnosed with pathognomonic genotypes in usherin

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Usher syndrome is an autosomal recessive disorder characterised by hearing impairment, retinitis pigmentosa (RP) and variable vestibular dysfunction. Usher syndrome presents both genetic and clinical heterogeneity, and is divided into 3 subtypes caused by at least 9 genes. Usher syndrome type II (USH2) is the most frequent clinical group. Affected patients show moderate to severe hearing loss (HL) and variable evolution of RP. Typical USH2 audiograms have a gently down-sloping configuration from moderate in the low frequencies to severe or profound in the high frequencies. High variability is recognised and clinical diagnosis of USH2 can be missed suggesting that USH2 is likely to be more frequent than estimated. USH2A is the overwhelmingly involved gene in USH2 and encodes two isoforms of the protein called usherin (a short isoform of 1546 residues and a long isoform of 5202 residues).

Audiograms were collected in a french cohort of 30 patients presenting with pathogenic genotypes in usherin. ISO 7029 norm-based calculation was used to abolish age-related and gender bias. For a third of these patients, audiograms could be collected over several decades in order to assess the progression of HL. Preliminary results tend to show a homogeneous range of hearing deficiency, either considering the affected isoform, or the type of mutations (truncating, missense or splice variants). However, some audiograms did not fit within the defined range; some of them could be explained by the presence, on one allele, of the controversial p.Cys759Phe variant.

#### P05.206

#### New mutations in VWF Gene, from mexican patients with „Von Willebrand Disease“

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We analyzed exon 28 of VWF gene from 20 Mexican Mestizo index cases with von Willebrand disease, using DNA amplification by polymerase chain reaction and direct sequencing using Big Dye (Applied Biosystem, USA). They have high frequency of blood group type O (80%), and normal or low aPPT, VWF:Ag, VWF:RCo, and FVIII:C. We found two novel mutations: (inst3706) and (delG4911) in patients with VWD type 1. Both produce an early stop with a short putative protein. The first one corresponding to a woman with menorrhagia, and the second one to a male with mucocutaneous bleeding to require hospitalization. Moreover, we found other polymorphisms previously informed. This is the second part of molecular study of VWF gene where we informed other three new mutations, previously.

#### P05.207

#### The study of the WFS1 gene is useful in non syndromic hearing loss, with ascending or U-shape audiometric curve

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**Introduction:** Wolfram syndrome or DIDMOAD is characterized by the association of diabetes, optic atrophy and hearing impairment. This autosomal recessive syndrome is due to *WFS1* gene mutations. Some dominant mutations of the same *WFS1* gene have been associated with nonsyndromic deafness, preferentially affecting low frequencies (DFNA6/14/38). Unlike the Wolfram's mutations, the dominant mutations are essentially located in the exon 8 of the *WFS1* gene.

**Methods:** We have analysed exon 8 of the *WFS1* gene in a cohort of 64 unrelated individuals affected by nonsyndromic hearing loss (HL). 23 individuals presented with an ascending audiometric curve and 41 presented with a U-shape curve.

**Results:** In four different families, we identified a heterozygous mutation: c.923C>G (p.Ser308Cys), c.1079G>A (p.Cys360Tyr), c.2141A>G (p.Asn714Ser) and c.2421C>A (p.Ser807Arg). These new *WFS1* variations segregate perfectly with the HL, are missense mutations affecting highly conserved residues, and are absent from 100 control DNA samples.

These 4 French families are affected with isolated autosomal dominant HL. In a same family, a mutation can cause an HL characterised by an ascending curve or a U-shape curve, and evolution between these two shapes has been observed. The exhaustive audiological and clinical examination of the 17 affected patients allowed us to precise DFNA6/14/38 phenotype.

**Conclusion:** The molecular screening of *WFS1* gene is useful for patients presenting with sporadic or familial non syndromic hearing loss, with an ascending or U-shape audiometric curve. Moreover, our study leads to clarify the spectrum of *WFS1* mutations and the related phenotype in non syndromic hearing impairment DFNA6/14/38.

#### P05.208

#### DNA repair efficacy determines the severity of XPB associated progeroid Cockayne syndrome

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Mutations in the basal transcription and nucleotide excision repair (NER) helicase XPB associate with a mild form of the combined cancer and premature aging syndrome xeroderma pigmentosum / Cockayne syndrome (XPCS). Due to the dual role of XPB and absence of animal models, the underlying molecular mechanisms of XPB-XPCS have remained obscure. Here, with the first Xpb mouse models we demonstrate that severe alterations in Xpb are lethal, providing explanation to the relative scarcity of XPB associated disease. Furthermore, we show that knock-in mice with an XPCS-patient-derived XPB mutation mimic the UV-sensitivity typical for XP, but fail to show CS features and age normally. However, further ablation of DNA repair capacity by crossings the Xpb mutant mice to other NER mutants causes appearance of CS-like features such as premature aging and increase in cellular sensitivity to chronic oxidative stress, demonstrating the causative role of DNA repair defect in the onset of XPB-associated accelerated aging. The Xpb/Xpa double mutants display a novel NER phenotype, with intermediate severity of ageing symptoms and remarkable inter-animal variance. This large variance provides experimental evidence to the hypothesis that stochastic DNA damage accumulation is an important determinant of clinical variance in NER syndromes. Based on our results, we suggest a model of NER phenotypes in which the inter-individual variation first increases and then decreases as a function of the degree of DNA repair deficiency.

**P05.209****A novel frame-shift mutation of the EDA gene in a Spanish family with X-linked hypohidrotic ectodermal dysplasia**

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X-linked hypohidrotic ectodermal dysplasia (XLHED) is characterized by defects in the development of ectodermal structures as sweat glands, hair and teeth. Mutations in EDA gene, which codes for ectopasins-A, cause alterations in the protein and seems to be responsible for this disorder. In the present study, we analyse, by PCR, heteroduplex analysis and automatic sequencing, the full coding region and the exon-intron boundaries of the EDA gene in DNA from peripheral blood of one Spanish family with XLHED.

We found an alteration in the EDA gene in two members of the studied family. This alteration consists in a duplication of 2bp (c.974-975ins-GA) in the 5 exon that produces a change in the DNA sequence of collagen like domain rich in Gly-X-Y motifs. This domain is important for multimerization of the protein. Furthermore this duplication produces a truncation of the last exon that codes for a part of a TNF-like domain, which can affect the NF-κB and c-Jun kinase pathway.

The two affected members of the family show remarkable phenotypic characteristics on skin, teeth, eyebrows, lips and head hair, but aggravated in one of them. This phenomenon is explained by inheriting of the mutation and epigenetics mechanisms of anticipation of the disease across generations.

This results show a novel mutation in the EDA gene in a Spanish family with XLHED syndrome which introduces a new reading frameshift and a premature stop codon. This extends our knowledge of pathogenic mutations in EDA gene.

**P05.210****X-linked juvenile Retinoschisis (XLRS) in Spanish patients: common ancestries, hot spots and incomplete penetrance**

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Purpose: X-linked juvenile Retinoschisis (XLRS) is one of the most common causes of juvenile macular degeneration in males, characterised by myrocystic changes, splitting within the inner retinal layer ("schisis") and presence of vitreous veils. The aim was to describe the genotype-phenotype correlation in Spanish XLRS families with mutations in the *RS1* gene.

Methods: The study was performed in 31 Spanish XLRS families, comprising 46 affected males and two symptomatic women. Molecular analyses included direct sequencing, haplotype construction and determination of the X inactivation pattern.

Results: 18 different *RS1* mutations were identified; eight of them were novel ones. These new changes included missense (p.Gln154Arg, p.Leu137Pro, p.Glu215Val, p.Arg197Ser, p.Pro192Leu, p.His207Asp), nonsense (p.Gln80Ter) and frameshift substitutions (p.Thr138fsX). 2 out of 31 "de novo" mutational events were detected. The most common mutation (p.Gln154Arg; 6/18) presented a common haplotype. The symptomatic women showed a normal X inactivation pattern. Interestingly, a healthy male presenting the p.Arg209His mutation was identified.

Conclusions: A prevalent mutation in Spanish XLRS patients (p.Gln154Arg) has been first reported in this work and presented a common origin. The frequency of "de novo" mutations was 6%, which mainly rise in CG dinucleotides (hot spots). Further analyses showed that the retinal affection in both women could be due to mutations in another gene. Nevertheless, the possibility of incomplete penetrance for *RS1* variants or a gain-of-function alteration in a different interacting gene might be suspected. Despite the mutation spectrum is large and the phenotype variable, there was no correlation between mutation type and severity of disease.

**P05.211****Hierarchical analysis of 28 Y-chromosome SNP's in the population of the Republic of Macedonia**

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Analysis of Y-chromosome haplogroups, defined by single nucleotide polymorphisms (SNP's), has become a standard approach for studying the origin of human populations and measuring the variability among them. Furthermore, Y-SNP's represent a new forensic tool, because their population specificity may allow to determine the origin of any male sample of interest for forensic purposes. The aim of this study was to develop a strategy for rapid, simple and inexpensive Y-chromosome SNP's typing in the population of R. Macedonia. We have studied a total of 343 DNA male samples; 211 Macedonians, 111 Albanians and 21 of other ethnic origin (Roma, Serbs and Turks). Methodology included multiplex PCR and single nucleotide extension reaction by SNaPshot multiplex kit. The set of 28 markers has been grouped in 5 multiplexes in order to determine the most frequent haplogroups using only 1 or 2 multiplexes. Twenty different Y haplogroups were determined among 343 male DNA samples. The finding that five haplogroups (E3b1, I1b1, J2b1a, R1a and R1b) comprise more than 70% of the Y chromosomes is consistent with the typical European Y chromosome gene pool. The distribution of the Y-haplogroups differs between Macedonians and Albanians. The most common Y haplogroup among Macedonians is I1b1 (27.5%), followed by three haplogroups present with similar frequencies E3b1 (15.6%), R1a (14.2%) and R1b (11.4%). Among Albanians the most frequent Y haplogroup is E3b1 (28.8%), followed by R1b (18.0%), J2b1a (13.5%) and R1a (12.6%).

**P05.212****Elucidating the molecular function of ZFYVE27 (Protrudin)**

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ZFYVE27 is a member of the FYVE-finger family of proteins. The FYVE-finger domain is suggested to be responsible for endosomal localization of these proteins and the majority of the FYVE-finger proteins serve as regulators of endocytic membrane trafficking. ZFYVE27 was identified as a spastin interacting protein and previously, we characterized its interaction with spastin in mammalian cells. More importantly, we identified a German family in which mutation in ZFYVE27 causes autosomal dominant form of hereditary spastic paraparesis (HSP). A comprehensive expression analysis of ZFYVE27 in mouse revealed a high level of expression primarily in the HSP affected tissues such as brain, cerebellum and spinal cord. Immunohistochemical analysis of tissue sections from various subdivisions of brain and spinal cord showed expression in both cell soma and axons of the motor-neurons. To elucidate the molecular function of ZFYVE27 *in vivo*, we are generating a loss of function mouse model by knockout strategy. Conceivably the phenotype of these mouse models might mimic the pathological features of HSP, therefore will provide us with a valuable model system to study the underlying cause for HSP etiology.

**P05.213****Pitfalls of mapping a large Turkish consanguineous family with vertical Monilethrix inheritance**

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Monilethrix, a rare autosomal dominant disease characterized by hair fragility and follicular hyperkeratosis, is caused by mutations in three type II hair cortex keratins. The human keratin family comprises 54 members, 28 type I and 26 type II. The phenotype shows variable penetrance and results in hair fragility and patchy dystrophic alopecia. In our study, Monilethrix was diagnosed on the basis of clinical characteristics and microscopic examination in a family with 11 affected members. Haplotype analysis was performed by three Simple

Tandem Repeat markers (STR) and *KRT86* gene was sequenced for the identification of the disease causing mutation. In the results of this, autosomal dominant mutation (E402K) in exon 7 of *KRT86* gene was identified as a cause of Moniltherix in the large family from Turkey.

#### P05.214

#### PEX7 gene mutation in infant with Rhizomelic Chondrodysplasia Punctata Type 1

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Rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive peroxisomal disorder. Clinically patients present dwarfism due to symmetrical shortening of proximal long bones, ataracts, periarticular calcifications, multiple joint contractures, and psychomotor retardation. So far three subgroups have been defined due to different genes involved. The most common of these is RCDP Type 1 which is caused by mutations in PEX7 gene.

Here we report a 4 year 6/12 month-old male infant diagnosed as RCDP due to distinct clinical manifestations: short stature, rhizomelic shortening of proximal long bones, multiple joint contractures, and psychomotor retardation. There was notable frontal bossing, depressed profile, a flat nasal bridge, dysplastic external ears, and small nares. Roentgenological studies included abnormalities such as severe shortening of the femur and humerus, irregular and broad metaphyses, calcific stippling of the epiphyses (humerus). Bilateral cataract was diagnosed later. Plasma phytanic acid levels were elevated. Mutation analysis for PEX7 gene revealed a homozygous mutation for 370\_396del127bp (del G124\_S132).

The parents were consanguineous and the family history yielded another similarly affected and deceased female child. After mutation analysis family was informed and data given for possibilities of a prenatal diagnosis in any subsequent pregnancy.

#### P05.215

#### Identification of a novel mutation in DKC1 in Russian family with Dyskeratosis Congenita

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Dyskeratosis congenita (DC) is a rare congenital syndrome characterized by the triad of reticular skin pigmentation, nail dystrophy and mucosal leukoplakia, and the predisposition to bone marrow failure and malignancies. Mutations in DKC1 gene encoding dyskerin are responsible for the X-linked DC. We found novel mutation in DKC1 in Russian family with X-linked DC. The proband was a 10-y-old boy with skin lesions, nail dystrophy, oral mucosa erosions, epiphora and blood marrow failure, and his 8-y-old brother had identical signs of ectodermal dysplasia without bone marrow failure. Their 2-y-old sister had no clinical signs of DC. Each of the 15 coding exons of the DKC1 gene and their flanking regions were amplified from genomic DNA by PCR and screened for mutations by SSCP analysis, and the shift detected on the SSCP gel was re-amplified, cloned and sequenced. The novel mutation is a 2-bp inversion in exon 3: NM\_001363:c.166\_167invCT (L55S). It was found in the proband and his brother; their mother was a carrier and the sister was not. This is the first report of DKC1 mutation analysis in Russian patients.

#### P05.216

#### Human developmental biology resource

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The Human Developmental Biology Resource (HDBR) is a unique resource, funded by the Wellcome Trust and UK-MRC to provide human embryonic and fetal tissue to the research community for gene expression studies. The HDBR is based at the Institute of Child Health, University College London and the Institute of Human Genetics, Newcastle University. The HDBR collects material from terminations of pregnancy. This valuable material is used primarily to study the ex-

pression of developmentally significant genes including genes implicated in birth defects and inherited metabolic disorders.

The HDBR has ethical approval for the collection, storage and distribution of material between 4 and 12 weeks of development. Ethics Committee approval and a laboratory Risk Assessment is required before material can be supplied. The HDBR can provide fresh, frozen or sectioned material. In addition the HDBR administers the Fetal Tissue Bank (FTB) collection, previously at the Hammersmith Hospital. The FTB collection is composed of material between 8 and 19 weeks of gestation and is either frozen or cryopreserved for the generation of cell lines. A significant proportion of the HDBR material is karyotyped and normal karyotyped material is provided for research but karyotypically abnormal material can be provided on request.

In addition the HDBR offers an in-house gene expression service for the analysis of RNA or protein using *in situ* hybridisation or immuno-histochemistry respectively. The HDBR provides electronic images for publication and advice on interpretation of results. Data is deposited, in a public database.

Data from recent HDBR studies will be presented.

#### P05.217

#### Renal damage triggers cyst formation in an inducible *Pkd1* deletion model

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is caused by a mutation in the *PKD1* or *PKD2* gene. This disease is characterized by large fluid-filled kidney cysts and a progressive deterioration of renal function eventually leading to renal replacement therapy.

We generated a tamoxifen-inducible, kidney epithelium-specific *Pkd1*-deletion mouse model. Deletion of the *Pkd1* gene in adult mice resulted in a mild cystic phenotype. However, in newborn mice, this results in massive cyst formation. In young mice, tubular cell proliferation still takes place to elongate the nephron, in contrast to the adult kidney. Therefore, we hypothesized that renal injury followed by a tissue repair response, including epithelial cell proliferation, accelerates cyst formation in adult *Pkd1*-deletion mice.

Inducible *Pkd1*-deletion mice were treated with the nephrotoxicant DCVC upon *Pkd1*-gene inactivation. 10-14 weeks after DCVC treatment, renal function rapidly declined in DCVC treated *Pkd1*-deletion mice, as determined by blood urea concentration. Kidney-to-body weight ratios were increased in DCVC treated animals compared to controls. Histopathological analysis revealed numerous cysts mainly of proximal tubular origin. Cyst formation was absent in DCVC treated *Pkd1*<sup>+/+</sup> mice while non-treated *Pkd1*-deletion mice only showed few focal cysts. Renal proliferation indexes were determined by Ki-67 staining at 1 wk after injury and were increased in *Pkd1*-deletion animals compared to DCVC treated *Pkd1*<sup>+/+</sup> mice.

In conclusion, these data provide evidence that injury-induced enhanced proliferation of renal epithelium in the absence of wild type polycystin-1 is a trigger for cyst formation. Since polycystin-1 localizes in the basal-body/centrosome, we hypothesize that aberrant planar cell polarity of the newly formed cells plays a role in ADPKD.

## P06. Genetic analysis, linkage, and association

### P06.001

#### Identification of novel variants in the Arylalkylamine N-acetyltransferase (AANAT) human gene and their contribution to Mood Disorders susceptibility

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Disruption of circadian rhythms, including abnormalities of circadian phase position and melatonin secretion, have been described in mood disorders (MD) but the molecular mechanisms underlying their pathology are largely unknown. Arylalkylamine N-acetyltransferase (AANAT) is a key enzyme involved in circadian oscillations of melatonin levels. In order to assess the possible contribution of AANAT gene variability to the susceptibility to MD, we systematically investigated common and rare variation in the AANAT gene through a sequential sequencing and single nucleotide polymorphisms (SNP)-based genotyping approach. Our sample consists of 445 unrelated patients with MD (257 unipolar major depressive disorder, 188 bipolar disorder) diagnosed according to DSM-IV criteria and 440 screened control subjects. The entire coding region, the exon-intron boundaries, the promoter and 3'UTR of the AANAT gene were directly sequenced in a subset of 360 MD patients by PCR, identifying 17 changes. Thirteen out of the 17 changes represent novel sequence variations and four had been described before: rs3760138, rs4238989, rs4646261 and rs8150. The novel and previously reported variants in dbSNP public database were genotyped in the rest of the MD sample and in the control sample using SNPlex genotyping system. Non-rare variants (MAF>2%) were further included in a case-control association study. The results showed significant differences in genotype distributions for two SNPs located in the promoter region of AANAT gene ( $p=0.00006$ ;  $p=0.004$ ), which remained significant after correcting for multiple comparisons. Our results suggest that genetic variability in the promoter region of AANAT gene contribute to the human susceptibility to mood disorders.

### P06.002

#### Frequencies of four ABCG8 polymorphisms in patients with ischaemic vascular diseases

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Background. ABCG5 and ABCG8 mediate sterol absorption and excretion. It is still not clarified how the most common polymorphisms of these genes contribute to cholesterol plasma level changes, and whether polymorphisms are associated with multifactorial vascular diseases.

Methods. We investigated 241 unrelated, consecutively enrolled patients with ischaemic stroke, 148 patients with coronary heart disease (CHD) and 191 blood donors as healthy controls for allele frequencies (AF) of four common ABCG8 polymorphisms (D19H, Y54C, T400K, A632V).

Results. Linkage disequilibrium test revealed linkage between the respective neighbouring loci of ABCG8. Estimated haplotype frequencies were similar for the three investigated groups (stroke or CHD patients and healthy controls). AFs of the investigated polymorphisms in patient-groups showed no significant differences compared to con-

trols. There was a tendency toward reduced YY54 genotype frequency in the entire stroke group. Upon stratification by age at disease onset, male stroke patients under the age of 50 (n=15) showed significantly reduced frequency of YY54 compared to control males (24.2% vs. 41.3%; OR:2.205 [95%CI:1.079-4.504]; p=0.0377). No such associations were found in female cases. In healthy controls, cholesterol levels of individuals with YY54 genotype (n=71; median 4.51 [25th-75th percentiles, 4.19-5.43] mM) were significantly reduced compared to YC54 and CC54 individuals combined (n=120; median 4.95 [4.42-5.88] mM, p=0.009). In addition, we identified a new ABCG8 variant, T401S, in a healthy control.

Conclusions. According to our data ABCG8 YY54 may be a potential protecting factor against ischaemic stroke in young males; and Y54C polymorphism may influence cholesterol plasma levels in healthy individuals.

### P06.003

#### Acetyl CoA carboxylase 2 (ACACB) promoter SNP -8414 C/T is associated with body fat in women and affects promoter activity

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Acetyl-CoA carboxylase (ACC) catalyses the formation of malonyl-CoA, an intermediate in fatty acid synthesis and a potent inhibitor of carnitine palmitoyltransferase (CPT1). CPT1 transfers long-chain fatty acyl-CoA (LCFA-CoA) to the mitochondria for  $\beta$ -oxidation, so ACC causes cytoplasmic accumulation of LCFA-CoA by increasing CPT1 inhibition. Elevated LCFA-CoA in the hypothalamus signals energy surfeit and leads to inhibition of feeding. We proposed that genetic variation influencing the level of expression of the ACC2 gene (ACACB) could be influential in determining body weight. We selected -8414 C/T (rs16939972), sited -368bp from the exon 1b transcription start site in ACACB promoter II, as a potential functional or marker site. We tested association with anthropometry, body fat, serum leptin, triglyceride and insulin sensitivity in 2633 healthy Caucasian females from the Twins UK cohort (mean age 47.3±12.6 y). Allele C was associated with higher total body fat ( $P=0.014$ ). We then tested the activity of the promoter with respect to -8414 C/T alleles in transfected HepG2 hepatocytes. The constructs contained a 907bp region (from position -870 to +37 relative to the transcription start site) and were co-transfected with an SREBP-1a expression plasmid. Activity associated with allele C was 57.5±4.0% and with allele T 25.0±10.8% of that of an SREBP-1a control plasmid, i.e. allele C showed 2.3 times greater activity than allele T. ACACB could be influential in determining body weight.

### P06.004

#### Polymorphisms in ACTN3, ACE and AMPD1 genes and physical performance in Bulgarian sub-elite athletes

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The aim of this study was to analyse ACTN3 (R577X), ACE (I/D) and AMPD1 (34C>T) polymorphisms in sub-elite athletes (n=70, 57 males and 13 females) and controls (n=44, 15 males and 29 females). The correlations between genotypes and physiological and biochemical parameters at anaerobic conditions was investigated. Athletes were divided into three sport groups according to a power-time model of performance intensity. The physiological parameters were evaluated by standard Wingate Anaerobic Test and Ergospirometry. Spectrophotometry and Blood-Gas analysis were used for the estimation of the glycolytic enzyme activity of Lactate Dehydrogenase and Acid-Base Balance, respectively. DNA samples were genotyped by RFLP analysis followed by agarose gel-electrophoresis. Differences in the distribution of alleles and genotypes between the groups were assessed by  $\chi^2$ -test. Statistical analysis of variances was performed using one way ANOVA. No significant differences between the athletes and controls

was found according the allele and genotype frequencies of the investigated polymorphisms. AMPD1 heterozygous male athletes in the "Anaerobic" group showed greater Mean Power Output (Watts) in comparison to CC homozygous athletes (9.11 vs. 7.34 Watts). Significant correlation was observed also with the buffering capacity (HCO3 and BE). No individuals homozygous for the T-allele of AMPD1 were found. The ACTN3 genotype correlated with parameters relevant to exercise capacity such as oxygen uptake, saturation and Lean Body Mass in the male sub-groups of anaerobic sports and endurance sports, but not in the female sub-groups.

#### P06.005

#### Case-control study of six genes asymmetrically expressed in the two cerebral hemispheres: evidence of association of *BAIAP2* and *NEUROD6* with adulthood attention-deficit hyperactivity disorder

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Attention-deficit hyperactivity disorder (ADHD) is a common childhood-onset neuropsychiatric disorder that persists throughout lifespan in at least 30% of ADHD children. Different lines of evidence suggest that abnormal right-left brain asymmetries in ADHD patients may be involved in a variety of ADHD-related cognitive processes, including sustained attention, working memory, response inhibition and planning. Although the exact mechanisms underlying cerebral lateralization are unknown, left-right cortical asymmetry in humans has been associated with transcriptional asymmetry at early embryonic stages and a number of genes differentially expressed between hemispheres have been identified. Among these, we selected six functional candidate genes showing at least 1.9-fold differential expression between hemispheres (*BAIAP2*, *DAPPER1*, *LMO4*, *NEUROD6*, *ATP2B3*, *ID2*) and performed a case-control analysis in 531 ADHD patients (320 children and 211 adults) and 531 sex-matched unrelated controls. The single- and multiple-marker analysis provided preliminary evidence for the contribution of *BAIAP2* ( $P=8.5e-06$ ; OR = 2.69 (1.74-4.17)) and *NEUROD6* ( $P = 0.0053$ , OR = 1.76 (1.18-2.61)) to adulthood ADHD. Additionally, association between both genes and performance deficits in the Conners Continuous Performance Test (CPT-II) was also identified. Our results support the participation of *BAIAP2* and *NEUROD6* in the continuity of ADHD across lifespan and suggest that genetic factors potentially influencing abnormal cerebral lateralization may be involved in the predisposition to this neurodevelopmental disorder.

#### P06.006

#### The genetic causes of sex differences in neurodevelopmental disorders

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ADHD and schizophrenia are heritable, neurodevelopmental disorders that are more prevalent in males. Both disorders also show sex differences in age of onset and severity. The Y chromosome is potentially an important influence on male susceptibility to neuropsychiatric disorders. Animal models have associated the Y chromosome with aggression and decreased levels of serotonin and dopamine in the brain. However, due to difficulties arising from the lack of recombination and widely accepted nomenclature, the Y chromosome has been largely excluded from genetic and genomic studies of neuropsychiatric disorders. In order to overcome this lack of knowledge we chose to study the Y chromosome in a sample of 210 cases with ADHD, 310 cases with schizophrenia and 700 U.K. controls. In total, 40 Y chromosome markers were selected to represent the main Y chromosomes

haplogroups that are present in the U.K. according to data from the Y chromosome Consortium data and personal communication with Y chromosome researchers. Statistical analysis of Y chromosome haplogroup analysis revealed no significantly increased representation of any haplogroup in cases with ADHD or schizophrenia compared to controls. However, this is one of the few studies to have genotyped Y chromosome markers in such a large number of U.K. individuals and therefore our results provide an insight into the population structure of U.K. Y chromosome haplogroups.

#### P06.007

#### The influence of adiponectin G276T gene polymorphism on changes in adiponectin levels or its oligomer composition by exercise training

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Purpose: Exercise training improves glucose and lipid metabolism mediated by altered adiponectin oligomer composition. The purpose is to determine the effects of exercise training on total and high molecular weight (HMW) adiponectin and adiponectin oligomeric distribution and influence of adiponectin G276T gene polymorphism on changes in adiponectin levels or its oligomer composition by exercise training.

Subjects and Methods: A randomized parallel-design study ( $n = 53$ ; 36 women and 17 men; aged 32-65 years) at a fitness club from April 2006 to July 2007 was conducted. Participants were randomly assigned to the exercise ( $n = 26$ ) or control ( $n = 27$ ) group and received exercise training for 70 min 2 times per week for 12 weeks and exercise advice at the baseline, respectively. Main outcome are muscle strength; body weight; body mass index; blood pressure; glucose and lipid parameter; circulating levels of total adiponectin and HMW adiponectin; and percentage of HMW adiponectin.

Results: After 12 weeks, there were no differences between the groups for the total adiponectin levels, HMW adiponectin levels, or percentage of HMW adiponectin. No significant difference in the change in the total and HMW adiponectin levels and the percentage of HMW adiponectin between the subjects with the G276G genotype and 276T allele carriers were found in either the exercise or control group.

Conclusion: In the absence of weight loss, Exercise training, does not change HMW adiponectin levels or the adiponectin oligomer composition. Adiponectin G276T gene polymorphism may not modify the adiponectin change response to exercise training.

#### P06.008

#### Genetic analysis of adult stature in Dutch isolated population

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We analysed a large complex pedigree from a Dutch genetically isolated population. About 2600 of 19700 pedigree members were phenotyped and genotyped for autosomal 5208 SNPs (Illumina 6K linkage panel). Complex segregation analysis of adult height was performed under mixed model including effects of biallelic major gene, polygene, age and sex. We used likelihood approximation based on breaking pedigree loops. The results confirmed large contribution of genes in the trait variance ( $h^2 = 0.85$ ) and significance of major gene effect in accordance with Elston-Stewart test. Three genotypic means were estimated as 183.5, 178.3 and 174.6 cm in males at 40 years with average difference of male and female genotypic means about 13 cm. The putative major gene explained 18% of trait variance.

A genome-wide scan was performed by variance-components method using Merlin program. Prior to analysis, the pedigree was split into smaller non-overlapping fragments, with maximum bit-size of 18. No loci demonstrated significant linkage, however for 6 loci linkage was suggestive:

SNP	Chr	Position(cm)	LodScore
rs1993104	19	56.9	2.71
rs1873191	18	44.7	2.60
rs1019845	2	195.8	2.27
rs958883	5	123.3	2.15
rs936347	16	17.2	2.11
rs216223	17	2.1	2.11

Of these six loci, five were identified in previous linkage analyses, while locus at chromosome 16 (rs936347) was new.

**P06.009****A candidate phenotypic modifier gene in fibrinogen deficient mice: beta-1,4-N-acetyl-galactosaminyl transferase 1 (galgt1)****R. J. Fish<sup>1</sup>, D. Vu<sup>1</sup>, A. Fort<sup>1</sup>, S. Deutsch<sup>1</sup>, J. Degen<sup>2</sup>, M. Neerman-Abez<sup>1</sup>**<sup>1</sup>*Geneva University Faculty of Medicine, Geneva, Switzerland, <sup>2</sup>Cincinnati Children's Hospital Research Foundation and University of Cincinnati College of Medicine, Cincinnati, OH, United States.*

In humans the absence of circulating fibrinogen leads to a bleeding disorder with variable severity, afibrinogenemia. Fibrinogen alpha chain knock-out mice (FGA<sup>-/-</sup>) are effectively afibrinogenic and show a strain-specific bleeding phenotype in neonates. This variability implies that modifier genes exist in FGA<sup>-/-</sup> mice and in humans with afibrinogenemia. About 70% of C57BL/6 FGA<sup>-/-</sup> mice die in the neonatal period whereas survivors have normal longevity. Fewer (~20%) FVB/N FGA<sup>-/-</sup> mice die as neonates but show high mortality at P30 to P60. To identify candidate FGA<sup>-/-</sup> phenotypic modifier genes, we used microarray analysis on liver RNA from both mouse strains. Of the genes identified with variable expression between strains, one encodes a Beta-1,4-N-acetyl-galactosaminyl transferase (galgt1). A galgt1 paralog, galgt2, was identified as a modifier gene in a mouse model of another human bleeding disorder, von Willebrand's disease(1). By RT-PCR, we found that galgt1 expression increases in the liver of C57BL/6 mice from P0 to P30. In contrast, galgt1 expression is low in FVB/N mice at birth and declines with age. Using strain-specific polymorphisms and sequencing of RT-PCR products, we determined the expression of C57BL/6 and FVB/N galgt1 alleles in livers from F1 hybrid mice. Both alleles were expressed at P0, but only the C57BL/6 allele was detected at P30. Using pyrosequencing, the FVB/N allele expression was 22.8% at P0 and undetectable at P30. We are investigating the genetic basis for this differential allelic expression and whether it can explain strain-specific phenotypic variation in FGA<sup>-/-</sup> mice. (1)Mohlke et al, (1999) Cell, 96, p111-120.

**P06.010****Identification of susceptibility genes for Multiple sclerosis: a study of the genomic region 2q13-14 in an Italian population****I. Borzani<sup>1</sup>, M. Tola<sup>1</sup>, L. Caniati<sup>2</sup>, G. De Santis<sup>1</sup>, A. R. Collins<sup>3</sup>, C. Scapoli<sup>1</sup>**<sup>1</sup>*University of Ferrara, Ferrara, Italy, <sup>2</sup>St. Anna Hospital, Ferrara, Italy, <sup>3</sup>University of Southampton, Southampton, United Kingdom.*

Understanding the genetic basis of multiple sclerosis (MS) remains a major challenge, despite decades of intensive researches. Whole genome linkage studies were carried out in different populations and from these analyses the prediction of at least 38 potential non-MHC susceptibility regions emerged (Abdeen et al., 2006). Among these, chromosomal portions of specific interest to some populations came out: the region 2q14 in the Irish and English population (Heggarty et al., 2003; Mann et al., 2002), the region 2q36 in the Sardinians (Corrado et al., 2003) and the region 2p21-22 in Continental Italian population (Liguori et al., 2003).

The main objective of this study was to investigate the role of 2q13-14 region in the pathogenesis of MS. The investigation was conducted on 120 patients, with clinically defined MS, and on 249 healthy subjects collected from the North-Italian population. Using CHROMSCAN (Maniatis et al, 2002, 2005), a map in LD-units, under the Malécot model for multiple markers, was constructed. The block-step structure of the 2q13-14 region was based on 70 SNPs (validated in the Caucasian population) selected from the HapMap database, with rare variant frequencies higher than 10% and evenly covering a region of 1.2 Mb within the segment 2q13-14.

We tested the association with the disease through both allelic association and by association mapping with the Malécot Model (Morton et al. 2007).

The present results support a possible association between this candidate region and MS in the North-Italian population.

**P06.011****Association between the N-acetyltransferase 2 (NAT2) gene polymorphisms and allergic rhinitis in Volga-Ural region of Russia****A. Khuzina<sup>1</sup>, A. Karunas<sup>1</sup>, A. Biktasheva<sup>2</sup>, A. Yuldasheva<sup>3</sup>, E. Etkina<sup>2</sup>, E. Khusnutdinova<sup>1</sup>**<sup>1</sup>*Institute of Biochemistry and Genetics, Ufa Scientific Center, Russian Academy of Sciences, Ufa, Russian Federation, <sup>2</sup>Bashkir Medical State University,**Ufa, Russian Federation, <sup>3</sup>Pediatric polyclinic N1, Ufa, Russian Federation.*

Allergic rhinitis (AR) is the result of an immunologically mediated hypersensitivity reaction of the nasal mucosa, initiated by exposure to specific allergens. The prevalence of AR in various countries ranges from 10-25%. It has been suggested in various studies that genetic defects in acetylation may be involved in the pathogenesis of allergic diseases and atopy.

The aim of this study was to examined the allele and genotype frequencies of three polymorphisms (481C>T, 590G>A, 857G>A) of the gene and analysed their combinations. The patient group consisted of 264 individuals with AR with different ethnic origins (Russians, Tatars, Bashkirs), the control group included 185 unrelated non-allergic individuals. The NAT2 alleles (\*4, \*5, \*6, and \*7) were determined by polymerase chain reaction-restriction fragment length polymorphism methods with DNA extracted from peripheral blood leucocytes by standard phenol/chloroform method.

The analysis has revealed that Tatars have significant higher frequency of \*4 allele (wild type) of the NAT2 gene in control group than in patients ( $P=0,0007$ ; OR=0,34; 95%CI=0,18-0,64). At comparison of group of patients with high total IgE level and the control group we have also determined increased frequency of \*4 allele of the NAT2 gene in healthy donors ( $P=0,026$ ; OR=0,53; 95%CI=0,3-0,93). Moreover, an association of \*5\*6 genotype of the NAT2 gene with AR at patients with high total IgE level has been found ( $P=0,012$ ; OR=2,4; 95%CI=1,19-4,82).

Thus, we have determined statistically significant association between NAT2 gene polymorphisms and allergic rhinitis in Volga-Ural region of Russia.

**P06.012****Inherited alopecia and ectodermal dysplasia in pakistani kindred****I. Ahmad, M. Tariq, A. Ali, M. Bakhtiar, A. Azhar, S. M. Baig;***National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan.*

Inherited Alopecia (AP) is a rare autosomal recessive disease clinically characterized by total or partial hair loss soon after their birth and the development of papular lesions of keratin-filled cysts over extensive area of body. The inherited hypohidrotic and anhydrotic ectodermal dysplasia (ED) is characterized by the absence or hypoplasia of hair, teeth and eccrine sweat gland. Ectodermal dysplasias are highly heterogeneous with more than 200 distinct clinical forms reported so far. The X-linked hypohidrotic ectodermal dysplasia (XLED) is the most common form of ED. In this study, twelve consanguineous families with alopecia and seven with ectodermal dysplasia were ascertained from Southern Punjab and Northern areas of Pakistan having multiple affected members. Pedigrees were analyzed to determine the pattern of inheritance; in four families with AP the disease was inherited in the autosomal recessive pattern. Short Tandem Repeat Markers (STR) were used in the exclusion mapping for the ten most common loci reported. According to results, one family with AP was linked to the type I keratin genes (17q21.2locus) while other families are still undergoing exclusion analysis. In four families with ED exclusion to all known loci has been observed whereas one family is showing linkage to the type II keratin (KRT) genes (12q13.13 locus). Exclusion analysis is in process in two pedigrees with ED. The families excluded to all known loci will be subjected to genome wide scan by SNP (Single Nucleotide Polymorphism) analysis using Affymetrix 250K array system to find the homozygous regions to find out the locus having the disease gene. Statistical analysis such as LOD (log of odds) will be used to validate the data obtained.

**P06.013****Alpha-synuclein in familial Parkinson's disease and Lewy Body Dementia****V. Greco<sup>1</sup>, E. De Marco<sup>1</sup>, F. Rocca<sup>1</sup>, P. Tarantino<sup>1</sup>, F. Annesi<sup>1</sup>, D. Civitelli<sup>1</sup>, G. Provenzano<sup>1</sup>, V. Scornaienchi<sup>1</sup>, I. Cirò Candiano<sup>1</sup>, S. Carrideo<sup>1</sup>, G. Squillace<sup>1</sup>, G. Nicoletti<sup>1,2</sup>, G. Annesi<sup>1</sup>**<sup>1</sup>*Institute of Neurological Sciences, National Research Council, Mangone (Cosenza), Italy, <sup>2</sup>Institute of Neurology, University Magna Graecia, Catanzaro, Italy.*

Alpha-synuclein has been implicated in the pathology of certain neurodegenerative diseases, including Parkinson's disease (PD) and de-

mentia with Lewy bodies (DLB). Alpha-synuclein is the major component of the filamentous Lewy bodies and Lewy neurites that define these diseases at a neuropathological level. The point mutations A30P and A53T in alpha-synuclein gene (*SNCA*) cause familial forms of PD and DLB. Recently, a third missense mutation E46K in *SNCA* was described in a Spanish family with DLB and parkinsonism. Moreover the A53T alpha-synuclein mutation was found in an elder Greek man with DLB. These cases suggest that E46K and A53T mutations should be considered in the differential diagnosis of DLB. The aim of this study was to evaluate the role of E46K alpha-synuclein mutation as a risk factor in DLB and in familial PD. We analysed the E46K mutation in seventeen sporadic DLB patients and thirty-seven familial PD patients. The clinical diagnosis of DLB was based on the criteria proposed by the International Consortium on DLB. PD patients were diagnosed according to UK Brain Bank criteria. We conducted a genetic analysis by standard PCR and restriction digestion method. None of the subjects examined had the E46K alpha-synuclein mutation. These results do not support a role for this mutation in our patients with DLB or familial PD, in agreement with the emerging consensus that mutations in the *SNCA* are associated with PD in few families worldwide.

#### P06.014

##### Associations of Alpha-1 antitrypsin Pi\*S allele with COPD

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Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive airflow limitation and related to an abnormal inflammatory response of the lung that results from a gene-environment interaction. The best documented genetic risk factor is severe hereditary deficiency of Alpha-1 antitrypsin (AAT). The presence of Pi\*Z allele (Glu<sup>342</sup>→Lys) in homozygote state, that is inherited in autosomal recessive way is already well proven factor for developing COPD. Data about Pi\*S allele (Glu<sup>264</sup>→Val) influence for developing COPD are not ascertain yet. Current study analyzed 1000 COPD patients, that clinical diagnosis was confirmed by using GOLD spirometric criteria. AAT serum concentrations were measured by means of nephelometry, and phenotyping was carried out by means of isoelectric-focusing. We found Pi\*S allele in 44 patients: 1 in homozygous state SS, 43 in heterozygous state: 40 MS and 3 SZ. AAT concentrations were significantly lower in SS and SZ group compared with normal AAT variant MM ( $p=0.035$ ). Lung function parameter - forced expiratory volume in one second (FEV1) was also worse in SS and SZ patients ( $p=0.043$ ) than in MM group. Clinical and biochemical characteristics of COPD patients with MS and MM phenotypes has no statistical differences. In conclusion, the results of the present study support the concept that Pi\*S allele is genetic risk factor for COPD for carriers of phenotypes SS and SZ, but not SM.

#### P06.015

##### Association of IL-6 with Alzheimer in a Brazilian sample

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Interleukin-6 is a multifunctional cytokine produced by immune and non-immune cells and act as an inflammatory mediator as well as endocrine and metabolic regulator. The IL-6 human gene is located in chromosome 7p21 and present many polymorphisms. The IL-6 rs 1800795 (-174G>C) polymorphism has been associated with Alzheimer's disease (AD) in some populations, however these findings have not been replicated in other studies. The IL-6 rs 1800796 (-572G>C) polymorphism has been considered as a risk factor for cardiovascular diseases and the IL-6 rs 00797(-597G>A) showed an association with systemic sclerosis, an autoimmune disease. To our knowledge, both polymorphisms have not been investigated in AD. DNA was extracted from blood samples of 302 subjects with similar ethnic origins, being 137 patients with AD, 130 elderly controls and 107 young controls. Subjects were genotyped concerning the three IL-6 polymorphisms using PCR-RFLP technique. AD patients were diagnosed according to NINCDS-ADRDA criteria. Association study was performed using Chi Square tests with  $p=0.05$ . No significant genotype distributions related

to -174 and -597 polymorphisms were observed among groups. However GG genotype and G allele of rs 1800796 polymorphism occurred more frequently in patients than in young controls ( $p=0.045$ ,  $p=0.031$ , respectively). A tendency to a higher frequency of GG genotype in elderly than in young controls was also detected ( $p=0.06$ ). In our study G allele excess among AD patients of rs 1800796 is due to either a problem of the control sample or associated to a AD susceptibility. Financial Support: FAPESP, CNPq, CAPES

#### P06.016

##### Association analysis of SNPs in PLAU and IDE genes with late-onset Alzheimer's disease in a sample of Mexican patients

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#### P06.017

##### Absence of Association between APOA1 Polymorphism and Alzheimer's disease

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Apolipoprotein A1 (APOA1) is the major apolipoprotein of the high-density lipoprotein, involved in reverse cholesterol transport. Alterations in cholesterol homeostasis influence the risk for Alzheimer's disease (AD). Vollbach et al. (2005) suggested that variation in the APOA1 might influence the function of the protein, and thus brain cholesterol metabolism, leading to an increased risk for AD. They identified two polymorphisms, a G/A substitution at position -75bp and a C/T and G/A base substitution at position -83bp or -84bp, or both, in the APOA1 promoter and investigated the effect of these polymorphisms on the risk for AD in 427 AD patients and 500 controls of German and English descent. They found that the A allele of the APOA1 -75bp G/A polymorphism was significantly associated with an increased risk for AD in subjects with an age at onset of 66 years or younger. In order to confirm these data we analysed the presence of this association in the Italian population recruiting 203 patients (n 75<=66 and 128>66 years old) affected by sporadic AD and 232 controls (n 100<=66 and 132>66 years old). In agreement with Vollbach et al. (2005), our analysis didn't

show any statistically significant association in LOAD group. On the other hand, we didn't find any association between APOA1 -75bp G/A polymorphism and the EOAD group ( $\chi^2=0.084$ , df = 2,  $p = 0.959$ ). In opposition to Vollbachet al. (2005) our results show that variants of APOA1 don't influence the onset and the risk for Italian AD sample.

#### P06.018

#### Paraoxonase gene polymorphism association with ALS in the French, Quebec and Swedish populations

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that results in the loss of upper and lower motor neurons. The disease is 90-95% sporadic in nature which suggests that multiple subtle genetic effects may lead to the development of ALS. One primary threat to motor neurons is oxidative stress. As such, an association has been observed between paraoxonase (PON) gene variants and ALS susceptibility. The PON cluster consists of three adjacent genes on chromosome 7q21.3 that aid in the detoxification of organophosphate insecticide and prevent the oxidation of lipoproteins. We sought to examine the frequency of coding and intronic PON polymorphisms in a set of 1197 ALS case and 1076 control samples from France, Sweden, and the founder French Canadian population of Quebec. Twenty SNPs that span the three paraoxonase genes were selected for Taqman genotype analysis. While individual SNPs were not considered associated on their own, a haplotype of SNPs at the C-terminal portion of PON2 that includes the PON2 C311S amino acid change was significant in the French (p-value 0.0075) and Quebec (p-value 0.026) populations as well as all three populations combined (p-value 1.69x10<sup>-6</sup>). Thus, we have identified two populations where susceptibility to ALS is significantly associated with variants in the PON gene cluster. The functional consequence of the variants identified with respect to motor neuron degeneration will provide insight into the role of these genes in susceptibility to ALS.

#### P06.019

#### Identification of TDP-43 variants in amyotrophic lateral sclerosis (ALS) patients

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Recently, TDP-43 was identified as a major component of ubiquitininated aggregates in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). We have screened 200 ALS patients, 120 sporadic (SALS) and 80 familial (FALS) cases and we have identified several missense variants in the TDP-43 gene. None of these variants were present in 360 unaffected controls. We are currently aiming to determine the causative effect of TDP-43 mutations in ALS pathogenesis and motor neuron degeneration. We will sequence further ALS cases for mutations, perform functional assays to determine what could be the gain or loss of function of TDP-43 and test whether this leads to selective vulnerability of motor neurons. Finally, we are generating three in vivo models, including transgenic mice, which will determine whether mutations in TDP-43 induce a motor neuron disorder and motor neuron pathology in other species. This project will contribute greatly to our understanding of ALS pathophysiology and the functional role that TDP-43 plays in this disease.

#### P06.020

#### Analysis of association of Apo E gene with placental insufficiency at habitual non-carrying of pregnancy

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Both non-carrying of pregnancy and placental insufficiency are the basis of many kinds of heavy obstetrical pathology which causes unfavorable outcome for the fetus.

The aim of this research is to study features of distribution of alleles of Apo E Gene at pregnant women of Kazakh population with clinical placental insufficiency and habitual non-carrying of pregnancy in anamnesis.

Materials and Methods of Research. By the method of PDRF analysis on the basis of PCR-mediated and site-directed mutagenesis, an analysis of frequencies of alleles of Apo E Gene was held at 12 pregnant Kazakh women with diagnosed placental insufficiency and habitual non-carrying of pregnancy in anamnesis. Examples of DNA, taken from 32 sound non-related pregnant Kazakh women with physiologically going pregnancy, were used as control examples.

Results. The analysis of frequency of detecting polymorphism of Apo E Gene made known that favorable genotype E2E3 was really detected more frequently at patients of the control group (75%) than at those of the basic group (50%). Frequency of genotypes E3E3 and E3E4 was 33.3% and 8.3% in the researched pathology group against 6.25 and 0% in the sound group. The revealed data are in accordance with data taken from publications, and they are proof of its possible genetic relation with unfavorable allele E3E4 of Apo E Gene at development of placental insufficiency during habitual non-carrying of pregnancy.

The further research of molecular-and-genetic markers will allow us to get more objective information about the nature and mechanisms for rise of placental insufficiency.

#### P06.021

#### A second candidate gene causing aniridia? : preliminary results

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Aniridia can range from a readily visible, almost complete absence of the iris, through enlargement and irregularity of the pupil mimicking a coloboma, to small slit-like defects in the anterior layer seen only on transillumination with a slit-lamp.

Approximately one third of all cases of aniridia are sporadic and these are often found to have cytogenetically detectable deletions involving 11p13, besides balanced chromosomal translocations and single chromosomal breaks.

Aniridia can also be caused due to six different categories of PAX6 mutations including nonsense, splicing, frame-shifting insertions or deletions, in-frame insertions or deletions, missense, and run-on mutations.

We found a large kindred with aniridia that have 10 live affected patients with autosomal dominant trait. While ophthalmological examinations of the affected patients revealed total aniridia, cranial magnetic resonance imaging studies showed no abnormalities in brain tissue. Karyotype analysis (550 bands) revealed normal male (46, XY) and sequencing of translated and untranslated exons of PAX6 gene were revealed no causative mutation. Chromosome 11 specific array-CGH for micro unbalanced translocation, deletion or amplification detection in or around the PAX6 gene is underway.

This preliminary results suggest that there can be another causative gene for aniridia. Whole genome linkage analysis is the next step to identify responsible chromosomal locus.

#### P06.022

#### APO E epsilon genetic polymorphism and foot ulceration severity in peripheral vascular complications of type 2 diabetes mellitus

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Peripheral vascular disease (PVD) includes inflammatory process that leads to cell damage and impaired regeneration within a vessel wall resulting in the formation of foot ulcers. PVD is a common and severe complication of type 2 diabetes mellitus. Many investigations show that polymorphisms in apolipoproteins genes can be associated with PVD.

Therefore, they may play a role in genetic predisposition of diabetic foot ulcers. In this study, 100 diabetic patients with PVD (age 55-75, disease manifestation 5-15 years) were divided into three groups using the Wagner ulcer classification system: the first group consisted of patients with 1-2 grade of ulceration, the second group with 3-4 grade of ulceration and the third group with 5 grade of ulceration. The allelic and genotype frequency distribution of *APO E epsilon* genetic polymorphism was estimated in these groups using the chi-squared test. We found out the allele and genotype frequencies in the second and the third group differed significantly from the same in the first group ( $p=0.05$ ). The diabetic PVD patients with 3 to 5 grade of ulceration had a significantly higher E4-allele frequency in comparison with the group of patients with 1-2 grade of ulceration.

#### P06.023

#### **Apolipoprotein A5 gene IVS3+G476A allelic variant confers susceptibility for development of ischemic stroke**

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**Background:** The T-1131C, T1259C and IVS3+G476A are naturally occurring variants of the apolipoprotein A5 gene; the aim of this study was to investigate the possible impact of these variants in the development of ischemic stroke. **Methods:** PCR-RFLP assays were used to determine the distributions of the apolipoprotein A5 gene alleles in small-, large-vessel and mixed subgroups of 378 patients and in 131 stroke-free control subjects. **Results:** Increased triglyceride levels were found in subjects carrying -1131C, 1259C, IVS3+476A alleles in all stroke groups and in controls. The -1131C and IVS3+476A alleles, but not the T1259C variant showed significant accumulation in all stroke subgroups. Logistic regression analysis adjusted for age, gender, BMI, total cholesterol levels, ischemic heart disease, hypertension, diabetes mellitus, smoking-and drinking habits revealed, that the IVS3+476A allele represent independent susceptibility factor for stroke (ORs for small-vessel: 4.748; large-vessel: 3.905; mixed: 2.926; overall: 3.644 at 95% CI;  $p<0.05$ ); we could also confirm the previously verified pathogenic role of -1131C variant. **Conclusions:** Our results suggest that while all of the three apolipoprotein A5 gene variants are associated with elevated triglycerides; only the -1131C and the IVS3+476A alleles confer risk for all types of ischemic stroke; such association could not be detected for 1259C allele.

#### P06.024

#### ***APOA5* gene S19W polymorphism and atherogenic dyslipidemia in patients with type 2 diabetes**

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Atherogenic dyslipidemia (AD), characterized by increased plasma levels of triglycerides or combined hyperlipidemia (hypertriglyceridemia and low plasma HDL level), is the most common in patients with type 2 diabetes (DM2). Apolipoprotein (apo) A5 gene S19W polymorphism has been shown to associate with increased plasma TG levels. So, the goal of this study was to define types of AD in patients with DM2 and to estimate the allele and genotype frequencies distribution of S19W *APOA5* in groups of patients with different types of AD. A total of 232 patients with DM2 (168 women and 64 men, mean age 57±7) were classified as belonging to a group with isolated hypertriglyceridemia (62 subjects), a group with isolated hypoalphalipoproteinemia (13 subjects), a group with combined hyperlipidemia (27 subjects) and a normolipidemic group (118 subjects). *APOA5* S19W genetic polymorphism was studied using the PCR-RFLP method. For statistical analysis of the allele and genotype frequencies distribution, the AD groups were compared with a normolipidemic group using the chi-squared test or the Fisher's exact test. We found out the allele and genotype frequencies in the group with isolated hypertriglyceridemia differed significantly from the same in the normolipidemic group ( $p<0.001$ ). At the same time there was a statistically significant difference in allele and

genotype frequencies between the patients with combined hyperlipidemia and the normolipidemic group ( $p=0.01$ ). The DM2 patients with hypertriglyceridemia both isolated and combined have significantly higher 19W-allele frequency in comparison with normolipidemic group of patients with DM2.

#### P06.025

#### **Autozygosity mapping in a large cohort of consanguineous Iranian families reveals three frequent loci for autosomal recessive intellectual disability**

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There is reason to believe that autosomal recessive intellectual disability (ARID) is more common than X-linked intellectual disability, but it has so far received considerably less attention. This is partly due to small family sizes and low consanguinity rates in industrialized societies, both of which have hampered gene mapping and identification. To shed more light on the causes of ARID and as a prerequisite for diagnosis, counselling and therapy, we have set out in 2003 to perform systematic clinical and molecular studies in large consanguineous Iranian families with several mentally retarded children. This has already led us to the discovery of 12 novel ARID loci, eight of which had a LOD score above three, and in one of them we were recently able to identify a mutation in GRIK2. Contrary to previous observations which *prima facie* argued against the existence of frequently mutated genes, investigations in our expanding cohort of more than 200 families have now led to the identification of three loci (on chromosomes 1, 5 and 19), that show overlapping autozygosity regions of three, four and six families, respectively. At each of these loci a minimum of two overlapping linkage intervals were solitary in the respective family and showed a significant LOD score of or above three. Mutation screening in order to elucidate the underlying gene defects is ongoing and likely to reveal frequent ARID genes. The outcome of our project will broaden the basis for genetic counselling and provide insight into the molecular basis of brain function.

#### P06.026

#### **Association of the methylenetetrahydrofolate reductase gene C677T polymorphism with unipolar depression**

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Unipolar depression (UD) is a complex disorder thought to result from multiple genes interacting with environmental and developmental components. The 5,10-methylenetetrahydrofolate reductase gene (MTHFR) is considered to be an important candidate gene of MDD. A single base mutation C677T results in production of a mildly dysfunctional thermolabile enzyme. The MTHFR 677T/T genotype and, to a lesser extent the 677C/T genotype, is associated with a significant elevation in the circulating concentrations of the homocysteine and a decrease in serum folate concentrations, which may be parallel a similar reduction in 5-methyltetrahydrofolate in CNS and may lead to reduction in monoamine neurotransmitter function and greater risk of UD. This study examined association of the MTHFR gene C677T polymorphism with UD in Russian and Tatar patients. Samples of 174 patients and 331 healthy volunteers were investigated using PCR method and subsequent enzyme digestion. There were significant differences in distribution of genotype ( $\chi^2 = 6.006$ ,  $P = 0.05$ ) and allele ( $\chi^2 = 4.603$ ,  $P = 0.03$ ) frequencies between depressive patients and controls in total samples. For the Tatars the MTHFR\*T/T (OR = 3.21, 95%CI = 1.04-9.93) and the MTHFR\*T (OR = 1.87, 95%CI = 1.19-2.94) are risk markers; the MTHFR\*C/C (OR = 0.51, 95%CI = 0.29-0.90) and MTHFR\*C (OR = 0.53, 95%CI = 0.34-0.84) are protective markers. For the Russians only MTHFR\*T/T genotype (OR = 2.56, 95%CI = 1.01-6.48) is a possible risk marker for development of UD. Our results confirm the role of MTHFR C677T polymorphism in susceptibility to unipolar depression in patients of Russian and Tatar descents.

**P06.027****The role of cytokine gene polymorphisms in the pathogenesis of childhood asthma**

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Asthma is a complex genetic disease characterized by increased airway responsiveness, reversible airway obstruction, and airway inflammation. Cytokines have important pathogenic role in this inflammation. In the present study, we analyzed associations between polymorphic variants of cytokine and cytokine receptor genes (*IL4*, *IL4RA*, *IL10*, *IL1B*, *IL1RN*) and asthma. The patient group consisted of 150 children with atopic asthma; the control group included 183 unrelated non-atopic individuals from Volga-Ural region of Russia. Genotyping was performed by polymerase chain reaction with specific primers followed by restriction digestion and gel electrophoresis.

A significant difference was observed in the allele and genotype frequencies of -627C>A polymorphism of the *IL10* gene and -590C>T polymorphism of the *IL4* gene between children with asthma and non-atopic control ( $p<0.05$ ). The frequency of CC genotype of the *IL10* gene was higher in asthma patients (58.0%) compared with control groups (42.1%; OR=1.9). Increased frequencies of TT genotype and T allele of the *IL4* gene were found in asthma patients (17.3% vs 7.7%; OR=2.5 and 46.0% vs 33.5%; OR=1.7, accordingly).

The analysis of -511C>T and 3953C>T polymorphisms of the *IL1B* gene, VNTR-polymorphism of the *IL1RN* gene, Ile50Val polymorphism of the *IL4RA* gene revealed that there are no significant differences in the frequencies of allele and genotype between asthma patients and control group ( $p>0.05$ ).

In summary, these results provided evidence for a role of *IL4* (-590 C>T) and *IL10* (-627 C>A) polymorphisms in susceptibility to childhood asthma in Volga-Ural region of Russia.

**P06.028****IL4RA and IL12B gene polymorphisms with atopic asthma and opisthorchosis**

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Two common polymorphisms of the human *IL4RA* (I50V) and *IL12B* (1188A/C) genes were studied in 150 Russian patients with atopic bronchial asthma (BA), patients with Opisthorchis felineus helminth invasion, patients co-affected by these two diseases, and 150 healthy controls from Tomsk (Russia). Unlike the previous studies, no association with any disease was found for *IL4RA* gene polymorphism. The 1188A/C exchange in the *IL12B* gene was not associated with BA as well; however, the 1188C/C genotype prevalence was significantly higher in opisthorchosis patients as compared to the controls ( $p<0.05$ ). These data suggest a possible involvement of common polymorphism of the *IL12B* gene in pathogenesis of opisthorchosis.

**P06.029****Association between genetic polymorphisms in transforming growth factor beta-1 (TGF $\beta$ 1) and asthma**

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Asthma is a complex disease resulting from a combination of multiple genetic and environmental factors. Transforming growth factor beta-1 (TGF- $\beta$ 1) is expressed in many cell types including inflammatory cells and structural cells, such as airway epithelial and smooth muscle cells. TGF- $\beta$ 1 may modulate airway inflammation and remodeling, therefore TGF- $\beta$ 1 is a plausible candidate gene for asthma.

To investigate association between asthma and polymorphisms of the *TGF- $\beta$ 1* gene, we conducted an age-sex matched case-control study including 283 asthmatic patients and 285 healthy controls in Taiwan. We selected 4 SNPs (rs1800469 C/T, rs1982073 T/C, rs2241715 T/G, rs2241716 G/A) on the *TGF $\beta$ 1* gene and performed genotyping

using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method or TaqMan assay.

There were significant differences in the genotype and allele distribution of rs1800469 and rs2241715 polymorphisms of the *TGF- $\beta$ 1* between asthmatic patient and controls. The combination of TC and TT genotypes of rs1800469 were also associated with a significantly increased risk of asthma as compared with the CC genotype [OR=1.697, 95%CI=1.151-2.503]. The G allele of rs2241715 was associated with an increased risk of asthma as compared with the T allele [OR=1.331, 95%CI=1.051-1.684], however, only borderline significant genotypic difference was found between asthma and control group. Significant difference in the observed haplotype frequencies between cases and controls was also found. Those subjects with haplotype TCTG have increased risk of asthma.

Our results suggest that the two polymorphisms of the *TGF- $\beta$ 1* gene are significantly associated with asthma in Chinese population of Taiwan.

**P06.030****Association between ABCA1 transporter mRNA level and atherosclerosis**

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ABCA1 transporter is known to play important role in the cholesterol transport from peripheral tissues. However its influence on atherosclerosis development remains not studied completely.

According to our hypothesis *ABCA1* gene expression level may influence on metabolism of antiatherogenic high density lipoproteins (HDL) and contribute to atherosclerosis development.

For *ABCA1* gene expression level analysis banks of cDNA, isolated from leukocytes and macrophages of controls and patients with atherosclerosis diagnosed using angiography, were created. A significant reduction of *ABCA1* mRNA level in leukocytes of patients with atherosclerosis when compared with controls was discovered. Mean *ABCA1* expression levels in leukocytes for the group of patients and for the control group are  $0.63\pm 0.28$  and  $0.92\pm 0.13$  ( $p=0.02$ ). In the same time we detected a significant increase of *ABCA1* mRNA level in macrophages of patients when compared with controls. Mean *ABCA1* expression levels in macrophages for the group of patients and for the control group are  $1.32\pm 0.11$  and  $0.90\pm 0.16$  ( $p=0.003$ ).

In the group of patients with atherosclerosis 2-fold increase of *ABCA1* gene expression level in macrophages when compared with the one in leukocytes was discovered. Mean levels of *ABCA1* gene expression in macrophages and leukocytes of patients are  $1.32\pm 0.11$  and  $0.63\pm 0.28$ , respectively, ( $p=0.000026$ ). In the control group *ABCA1* gene expression level in macrophages did not differ from the one in leukocytes.

The study was supported by Russian Foundation for Basic Research (grant 06-04-49609).

**P06.031****Study of heme oxygenase 2 gene mutation as risk factor for CAD in Iranian patients with Atherosclerosis**

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Heme oxygenase 2 (HMOX2) enzyme is important in oxidative stress found in endothelial cells and adventitial nerves of blood vessels. This enzyme in collaboration with heme oxygenase 1 enzyme metabolizes heme molecules into ferrous iron, carbon monoxide (CO) and biliverdin which is further reduced to bilirubin. Both of biliverdin and bilirubin are potent antioxidants and reduce the chance of atherosclerosis. HMOX2 induces endothelial relaxation by synthesizing CO. This is the first study to investigate heme oxygenase 2 gene mutations in 137 patients with premature CAD affected with atherosclerosis and 100 normal controls. Pairs of primers have been designed to amplify exons 2, 3 and 4 of HMOX2 gene. All products have been analyzed by SSCP analysis and shifted fragments were separated for further sequencing. Two new sequence variations were observed among 13 patients with atherosclerosis consisted of C to A substitution in codone A70D (GCC to GAC) and A to G substitution in codone K89E (AAG to GAG). Levels of total direct and indirect bilirubin were determined in patients and

control groups. Statistical analysis showed significant association between A to G mutation and risk of atherosclerosis ( $P = 0.01$ ). No significant alteration in the level of total bilirubin was observed between case and control groups ( $P = 0.6$ ). This is the first report on the association between HMOX2 and atherosclerosis among Iranian CAD patients. This finding presences the importance of this mutation in development of atherosclerosis. More study would show the importance of hemoxygenase 2 gene mutation in other populations.

#### P06.032

##### Large scale association study of gene-gene interaction within the filaggrin pathway

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Filaggrin deficiency due to null mutations in the *FLG* gene has been established as risk factor for atopic eczema (AE). Processing of pro-filaggrin to biologically active filaggrin monomers involves several dephosphorylation and proteolytic steps, and their impairment might also disturb skin barrier function. Among the proteases suggested to be implicated in profilaggrin processing is the stratum corneum chymotryptic enzyme (SCCE), which is possibly regulated by *SPINK5*-derived serine proteinase inhibitor LEKTI. An insertion in the 3' untranslated region of the kallikrein 7 gene (*KLK7*) encoding SCCE as well as a *SPINK5* variant have been reported to be associated with AE, but could not be replicated so far.

In our study we aimed at clarifying the role of these genetic variants for AE. Considering the potential biological interactions between filaggrin, SCCE and LEKTI, we also examined gene-gene interactions.

Association analysis was carried out in a cohort of 486 German families as well as in a cohort of 773 cases and 2924 supernormal controls. Whereas the strong effect of *FLG* polymorphisms was confirmed, no association of the *KLK7* insertion could be detected. Concerning the *SPINK5* polymorphism rs2303067 A-allele, weak association was observed in the family collection only, with a strong maternal effect. There was no evidence for epistatic effects between *FLG*, *KLK7*, and *SPINK5* variants that significantly predict AE risk. Thus, our data confirm the impact of filaggrin deficiency due to *FLG* loss-of-function mutations on AE risk, but do not support the hypothesis that this effect is dependent on *KLK7* or *SPINK5*.

#### P06.033

##### Linkage and linkage disequilibrium scan for autism in an extended pedigree from Finland

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As a part of the genetic study of autism spectrum disorders (ASDs) in the Finnish population, an extensive genealogical search was conducted to find out whether a substantial fraction of the families would share the same ancestral lineage. Based on this search back to the 17th century, 18 ASD families (33 affected) were merged into one extended pedigree.

We hypothesized that this pedigree could expose rare ASD gene(s) enriched to this internal subisolate, and performed a genome-wide scan using 1107 STS-markers (intermarker spacing ~3.4cM). A joint analysis of linkage and linkage disequilibrium was performed with Pseudomarker statistics and a traditional multipoint linkage analysis with Simwalk2.

Nine loci exceeded the chosen significance level of  $-\log(p)>2.5$ . Of these, 1q21-23 ( $p=0.00082$ ) is one of the best loci in our previous genome-wide scans for autism and Asperger syndrome in Finland, while 15q11-13 ( $p=0.00081$ ) is a well-recognized site for cytogenetic abnormalities associated with autism. Best multipoint linkage was observed at 19p13 [ $-\log(p)=3.57$ ].

Regional candidate genes were chosen from these best loci at 1q23, 15q12 and 19p13, and analyzed with SNPs using additional 126 families with ASDs (281 affected). Most significant association was

observed with five consecutive SNPs of *ATP1A2* (1q23;  $p=0.00055$ ) and with eight consecutive SNPs within a *TLE*-gene cluster (19p13;  $p=0.000078$ ). This association evidence was not detected using the nationwide sample, suggesting enrichment of these loci to our pedigree. We are currently performing a high-resolution analysis of the extent of shared chromosomal regions among the members of the pedigree with Illumina 317K platform.

#### P06.034

##### The BDNF receptor gene *NTRK2* is a susceptibility gene for autism

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Autism is a complex neurodevelopmental disorder likely determined by multiple genes. The *NTRK2* gene encodes a receptor, TrkB, for neurotrophin 4/5 and Brain-Derived Neurotrophic Factor (BDNF), which promotes neuronal differentiation and regulation of synaptic function in the developing and adult nervous system. Intriguing observations, such as increased BDNF levels in autistic children and *NTRK2* mutations in children with developmental delays, raise the hypothesis that an abnormal function/expression of *NTRK2* might be involved in autism. 38 tag SNPs, spanning the *NTRK2* gene, were tested for association with autism in 323 Portuguese trios. We found significant transmission disequilibrium of alleles at three markers in *introns* 5-6 and 15-16 ( $0.011 < P < 0.041$ ). Given this evidence for association, we genotyped these markers in a second population sample of 168 Irish trios. In the combined sample (N=491) we found an association of the same three SNPs at a more stringent *P*-level ( $0.006 < P < 0.015$ ), and found nominal associations with four additional markers in *intron* 22-23 ( $0.0211 < P < 0.043$ ). Previously we have described a significant increase of plasma BDNF levels in approximately 30% of our patients. We therefore screened *NTRK2* exons encoding ligand-binding and catalytic regions (exons 6, 7, 12, 18, 20-24) for mutations in 20 autistic individuals with increased BDNF levels and 10 controls. No mutations were identified in these individuals, and there was no evidence for association of *NTRK2* with plasma BDNF distribution. The overall results therefore suggest that *NTRK2* may be a susceptibility gene for autism, but its impact on autism is not mediated by the increased BDNF levels.

#### P06.035

##### A large-scale systematic search for causal variants involved in autism and schizophrenia: resequencing of X chromosome synaptic genes

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Autism (AUT) and schizophrenia (SCZ) are two common neurodevelopmental disorders, which result from the combination of genetic and environmental factors. Linkage studies on the whole genome and association studies with candidate genes have failed to clearly identify the genes involved in the pathogenesis of these two diseases. We hypothesize that several different rare variants in numerous genes, including *de novo* variants, could lead to these diseases.

Therefore, we decided to directly sequence, in 285 AUT and SCZ patients, genes coding for proteins involved in the synapse, as defects in synaptic processes can lead to impairment in cognitive function. We decided to focus on the X chromosome, as evidence supports its implication in the predisposition to AUT especially, but also to SCZ.

Using various methods and sources, we established a complete list of 183 synaptic and potentially synaptic genes located on this chromosome and we ranked them according to their relevance for the diseases. We selected in this way 104 X-linked candidate genes that have been sequenced in our patient cohort. We have already identified more than hundred rare non-synonymous variants in AUT and SCZ patients. Some of them are particularly interesting due to their inheritance mode and their potential effect on the protein.

The screening of synaptic genes located on the X chromosome has led

to the identification of deleterious variants that could be responsible for AUT or SCZ in our patients. We are currently performing functional validation of these variants using animal models (zebrafish, fruitfly, worm or mice neurons).

#### P06.036

#### Association between polymorphisms of immune defense modifier genes and autoimmune diseases in Tomsk population

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The results of an investigation of the influence of immune defense modifier genes polymorphism [*IL1B* (+3953A1/A2), *IL1RN* (VNTR), *IL4* (3'-UTR G/C), *IL4RA* (I50V), *IL12B* (A1188C) and *VDR* (F/f and B/b)] on the development and presentation of type 1 diabetes, celiac disease (CD), and autoimmune thyroiditis (AT) are presented. The study was performed in 49 families with 139 patients affected by CD, 110 families with 350 type 1 diabetes patients, 119 patients with AT, and 129 unaffected controls of Russian ethnicity from Tomsk. We discovered various potential associations. The first was between +3953A1/A2\**IL1B* and type 1 diabetes ( $p=0.040$ ), and the second between the VV genotype of I50V\**IL4RA* and AT ( $p=0.032$ ). Family-based studies revealed association between 3'-UTR G/C\* *IL4* and CD ( $p=0.024$ ), and between I50V\**IL4RA* with type 1 diabetes ( $p=0.018$ ), and additionally with complications of the disease: diabetic retinopathy ( $p=0.050$ ), nephropathy ( $p=0.026$ ) and neuropathy ( $p=0.050$ ). Furthermore, the association with clinical course of the CD (typical form) was obtained for I50V\**IL4RA* and F/f\**VDR* polymorphisms ( $p=0.001$  and  $p=0.009$ , respectively). The combination of type 1 diabetes and AT was associated with allele A2 of the VNTR\**IL1RN* polymorphism ( $p=0.018$ ), whereas combination of CD and AT was associated with allele C of G/C 3'-UTR\**IL4*. Thus, in this investigation we detected associations of the studied phenotypes mainly occurred in polymorphic variants of the Th2-immunity genes.

#### P06.037

#### Genetic analysis of pedigrees with autosomal dominant Premature Graying of hair

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Whiteness of hair is one of the most recognizable early signs of aging. This is also termed 'Canities'. It is caused by the gradual decrease of pigmentation that occurs when melanin ceases to be produced in the hair root, and new hairs grow without pigment. The change naturally occurs as people age, usually turning hair from its natural color to gray, then to white. Premature-graying of hair (MIM 139100) or whiteness of the hair is a phenotypic change that appears as early as at teens and twenties, for some, even in childhood. The causes of this relatively common condition are largely unknown, although genetic, medical and other environmental factors all are suspected. The phenotype is frequently associated with many known genetic disorders such as Book syndrome, Waardenburg syndrome and Lison syndrome. The most common factors that could trigger the condition were tobacco smoking. We have studied six large Indian families with premature-graying of hair. The phenotype in these families is constituent with autosomal dominant mode of inheritance. The pedigrees consist of 290 individuals including 57 affecteds (25 males 32 females). The age of onset ranged from early childhood to teenage with a graying range from 20-40% hair and it increased with the advancement of age. Skipping of a generation was observed in three pedigrees. Cytogenetic analysis of two affecteds in each family did not show any anomaly. Systematic genome-wide association or high density linkage studies with microchips could be initiated in these families to identify the genetic loci. Email: u\_c\_rao@hotmail.com

#### P06.038

#### A strong association of axillary osmidrosis with genotype of the ABCC11 gene defining the earwax type

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Two types of cerumen are known in humans: the wet type with brownish sticky earwax, and the dry type lacking or reducing ceruminous secretion. The wet type is predominant in populations of European and African origin, while the dry type is often seen in Eastern Asian populations. An association of axillary odor with the wet earwax type among the Japanese was reported about 70 years ago. However, the data of the two traits were based on phenotypical analysis by researchers or on self-declaration by the subjects examined, because of lacking of definite diagnostic methods. We recently found that a SNP (rs17822931) of the ABCC11 gene is the determinant of the earwax types. In the present study, a total of 79 individuals with axillary osmidrosis (AO), who received a surgical operation to remove bilateral axillary apocrine glands, were examined for their earwax types by genotyping at the rs17822931 polymorphic locus. The earwax-type frequency among them was compared with that in the general Japanese population. The result showed a strong association between AO and the wet earwax type ( $c2 = 90.00$ ,  $p < 2.5 \times 10^{-21}$ ). In addition, immunohistochemical study of the axillary and ceruminous apocrine gland tissues using an anti-ABCC11-protein antibody revealed that ABCC11 is strongly expressed and localized in the apical membrane of the both gland cells. The result indicates that ABCC11 protein (MRP8) functions in the axillary apocrine gland as well as in the ceruminous gland, and supports the association between axillary odor and earwax type.

#### P06.039

#### BCL1 glucocorticoid receptor gene polymorphism and bone mineralisation and metabolism in juvenile idiopathic arthritis

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Juvenile idiopathic arthritis (JIA) is group of chronic inflammatory joint disease with different complications, such as osteopenia. Some patients receive only nonsteroid anti-inflammatory drugs (NSAID) and other patients receive glucocorticoids.

The aim of our study was whether *Bcl1* glucocorticoid receptor (GCR) gene polymorphism associated with bone mineralization and metabolism disturbances in JIA children.

Objectives: we included in our study 122 JA children, 43 boys (35,2%) and 79 girls (64,8%). Glucocorticoids were administered 30 children (24,6%), 3 boys and 27 girls. Another 92 JIA children were treated with NSAID.

Methods: *Bcl1* GCR polymorphism was detected by polymerase chain reaction with restriction assay [Fleury I. et al, 2003]. Osteopenia was detected by dual-energy X-ray absorptiometry L1-L4 (Hologic QDR 4500C), with national pediatric referent database (Scheptyagina L.A., 2005 et al). Bone metabolic markers, such as total Ca, Ca<sup>++</sup>, phosphate, and total alkaline phosphatase, osteocalcine, β-CrossLabs and parathyroid hormone also were detected in our patients.

Results: We haven't differences between genotypes and alleles distribution between children, who received NSAID and glucocorticoids. Girls with osteopenia ( $Z\text{score} < -1,0$  SD) had significantly higher GG genotype and G allele GCR gene. Girls, carriers G allele had significantly low bone mineralization parameters: bone mineral density (BMD) - Z score ( $p=0,003$ ) and tendency: bone mineral content ( $p=0,07$ ) and BMD, measured in g\sm<sup>2</sup> ( $p=0,06$ ) and significantly high

osteocalcine level ( $p=0.05$ ) when girls with CC genotype. Conclusion: in our study we have revealed that GG genotype and G allele *BclI* GCR was associated with osteopenia in JIA girls with high osteocalcine level.

#### P06.040

#### S2G (Syndrome to Gene): Novel software for the identification of genes associated with human genetic syndromes

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The identification of genomic loci associated with human genetic syndromes has been significantly facilitated through the generation of high density SNP arrays. However, the identification of the specific disease-associated genes within such loci is still a tedious labor-intensive bottleneck. Optimal selection of candidate genes from within a defined genomic locus is a crucial step in the process.

We have generated novel software that selects optimal candidate genes, using a two step procedure. For any given syndrome, the software seeks all genes associated with syndromes containing similar phenotypes. It then searches through the entire human genome for other genes that are associated with these reference genes. This search takes into account interacting biochemical pathways, protein-protein interactions, transcription factor cascades, etc. A gene list is generated with an order of priority (degree of possible interaction with the reference gene(s)). This list is then filtered for the specific genomic locus in question - and a prioritized list of candidate genes is generated.

S2G can be used also in clinical genetics: when S2G gets a query for related syndromes of a given one, the score vector of the given syndrome is sorted creating a list of syndromes starting with the closest related syndrome. The result of the query is a list of syndromes with their known causing genes, so one can view and explore which genes are associated with similar phenotypes to those of the query syndrome.

#### P06.041

#### Dystrobrevin binding protein 1 gene (DTNBP1) in a Bipolar Case-Control Study (BACCS)

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Background: Recent studies suggest a substantial degree of overlap in genetic susceptibility across the traditional categories of schizophrenia and bipolar disorder. There is consistent evidence for an association of the DTNBP1 gene with schizophrenia, and thus this gene has also become a focus of further investigation in bipolar disorder (BD).

Methods: The aim of our study is to explore the association of DTNBP1 gene with BD and with a sub phenotype such as presence/ absence of psychotic symptoms in a sample of 465 patients with BD (ICD10/DSMIV) and 478 ethnically matched control subjects recruited from the UK. Seven SNPs of the DTNBP1: rs2743852 (SNP C), rs760761 (P1320), rs1011313 (P1325), rs3213207 (P1635), rs2619539 (P1655), rs16876571 and rs17470454 were investigated using SNPlex genotyping system.

Results: We report significant differences in genotypic and allelic frequencies of rs3213207 and rs760761 of DTNBP1 gene between the bipolar patients and controls. We also show a global haplotypic association and an association of a specific haplotype within this gene with BD.

Conclusions: Our results are consistent with previous studies in term of a general association between the dysbindin gene and bipolar disorder and provide additional molecular genetic evidence that a portion of the genotypic overlap between schizophrenia and bipolar affective disorder is attributable to this gene.

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#### P06.042

#### A 7 Mb region within 11q13 is candidate to contain a high penetrance gene for breast cancer

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Familial breast cancer represents up to 5% of the total breast cancer cases. Only a fraction of families affected by breast cancer is explained by mutation in *BRCA1* and *BRCA2*. Recently, our group has performed a new SNP-based linkage study in 19 non-BRCA1/2 families from Spain, Netherlands and USA. We found that a single family was linked to two different chromosomes (regions 11q13 and 14q21), and showed a non-parametric LOD score of 11.5 in both regions. The regions spanned 28 and 14.5 Mb respectively. We ruled out any possible translocation between both chromosomes using cytogenetic techniques. We used both a panel of STRs and an indirect approach based on HapMap data to narrow down these two regions from 28 to 7Mb in chromosome 11 and from 14.5 to 8.5 Mb in chromosome 14. We selected the 11q13 region to perform a mutational screening on some candidate genes (*NuMA1*, *FGF3*, *CCND1*, *RAD9A*, *RNF121*, *FADD* and *has-mir-192*). Although we have not found any deleterious mutation in the coding sequence of these genes, data from infrequent markers located in different genes confirms 11q13 as a candidate region to contain a breast cancer susceptibility gene. A larger study involving new families could limit their size and facilitate a more extensive mutational screening.

#### P06.043

#### *PPP3R1* and *NFATC4* polymorphisms have no influence on cardiac parameters in healthy individuals and related to cardiac remodeling in patients with cardiovascular disease

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Numerous data appeared supporting the notion about an important role which calcineurin pathway plays in myocardial hypertrophy, cardiac remodeling and human heart failure. Though only few studies had been reported to date which demonstrate an association of calcineurin pathway genes polymorphisms with cardiac parameters or other cardiovascular measurements.

In this study we analyzed association of two calcineurin pathway genes polymorphisms (5-base pair deletion in calcineurin B promoter - 5/15D *PPP3R1* gene polymorphism and SNP rs2228309 in exon 2 of *NFATC4* gene - G160A) with echocardiographic parameters in 216 healthy individuals. Two polymorphisms under study had previously been shown by us to be associated with left ventricular (LV) remodeling in patients with arterial hypertension.

In healthy subjects we did not find association of those two polymorphisms with LV myocardium mass index, wall thickness, LV remodeling index, end diastolic size, ejection fraction or other cardiovascular parameters.

To further analyze the role of two functional genetic variants we collected a group of patients with ischemic heart disease and ischemic cardiomyopathy (n=104). We had revealed that patients differed from healthy subjects in *A160G NFATC4* gene allele frequencies ( $p=0.046$ ).

Our data supports the assumption that calcineurin pathway components may be involved in pathological types human heart remodeling.

#### P06.044

#### Study of genetic risk factors for cardiovascular disease in São Miguel Island population (Azores)

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Several genetic variants – factor V Leiden (G1691A), prothrombin (G20210A) and *MTHFR* (C677T and A1298C) – were found to be associated with cardiovascular disease (CVD), a frequent cause of death in many populations. The aim of this study is to investigate the preva-

lence of CVD risk mutations in São Miguel Island (Azores). From this population, 87 healthy individuals were analysed for the following variants: factor V Leiden, *MTHFR* (C677T and A1298C) by PCR-RFLP, and prothrombin by real-time PCR (FACTOR II Q - PCR Alert Kit, Nanogen Advanced Diagnostics). The allelic and genotypic frequencies of these polymorphisms were estimated and their values were compared with other populations. The results demonstrate that values of the mutant alleles *MTHFR* 677T (38.5%) and 1298C (23.6%) were relatively similar to those found in other populations. However, in São Miguel Island population, factor V Leiden shows a value of 1.7% for the 1691A allele, one of the lowest in Europe. Whereas, prothrombin (20210A) is present at a frequency of 3.4%, being one of the highest. Genotype frequencies of the genetic polymorphisms analysed showed similar values to other European populations. Furthermore, thirteen genetic profiles were identified in the study group: 13 individuals (14.94%) had none of the variants, 37 (42.53%) only one, 33 (37.93%) two, and 4 (4.60%) had three or more polymorphisms. The joint analysis of these polymorphisms represent a valuable contribution to further understanding the CVD in São Miguel Island. Funded by Azorean Government (M1.2.1./I/003/2005). Imotavieira@hdes.pt

#### P06.045

##### Analysis of sequence variants and splice isoforms in IL18RAP in celiac disease

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Celiac disease (CD) is a common autoimmune disorder of the small intestine induced by the ingestion of gluten protein in genetically susceptible individuals. CD is a complex genetic trait in which presence of the HLA-DQ2/8 genotype is necessary but not sufficient for the disease development. Previously a genome wide association study (GWAS) in the UK celiac cohort initially identified a new non-HLA locus on chromosome 4q27. In a more extensive follow-up study, 1020 UKGWAS top associated SNPs were independently genotyped in three additional European celiac cohorts (2,410 celiac cases, 4,828 controls) and seven novel celiac loci were identified. One of the associated regions ( $P$  overall=8.49x10<sup>-10</sup>) maps to a 350-kb block on the chromosome 2q11-2q12. This block contains four genes including two IL-18 receptors (*IL18R1* and *IL18RAP*). The strong correlation between the *IL18RAP* mRNA expression and associated SNP was observed in a group of treated celiac patients ( $p<0.0001$ ). Since the associated SNP is an intronic variant we sequenced the coding regions of *IL18RAP* in 23 celiac disease patients and 8 controls, and found 19 variants, 17 of which were already in dbSNP. One novel variant (c.1210+17A>G) was observed in a single control and the other one (c.1384+70\_1384+71insT) was found in two celiac individuals. We are now genotyping the 19 SNPs in a large cohort of Dutch, UK and Irish celiac samples. *IL18RAP* is known to express two splice variants. Using quantitative real-time PCR we are currently testing for correlation between the genotypes and the expression of *IL18RAP* splice isoforms.

#### P06.046

##### Relationship between Beta (3) Integrin (ITGB3) Leo 33 Pro Polymorphism and cerebral infarction

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Objective: To explore the distribution of B beta 3 integrin (ITGB3) polymorphism in Kazakh population and the association of the polymorphisms with the occurrence of different types cerebral infarction.

Methods: The B beta 3 integrin - Leu 33 Pro polymorphism was identified by polymerase chain reaction and restriction fragment length polymorphism in 37 patients with different cerebral infarctions (CI) and 87 healthy controls matching on age and sex..

Result : Distribution of genotypes ITGB3 basic group ( LL 37,8%, LP 56,8%, PP 5,4%),in control group (LL 55,2%, LP 44,8%) ( $p=0,2880$ ).

Frequency 33Pro allele provided of above that control group (33, 8% and 22, 4% accordingly) ( $p<0,05$ ).

Conclusion: The study demonstrated that 33 Pro allele of the B beta

(3) integrin gene (ITGB3) -Leu33Pro polymorphism may be a susceptible predictor different types of the cerebral infarctions in Kazakh population.

#### P06.047

##### Analysis of N-acetyltransferase 2 gene polymorphism in cervical cancer

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Cervical cancer (CC) is one of the most common causes of morbidity and mortality in women. Although the HPV is a major factor associated with CC the other risk factors have been indicated including smoking, oral contraceptive usage, lack of awareness for preventive care as well as genetic risk factor.

In our research N-acetyltransferase 2 (NAT2) gene polymorphism was estimated as a possible genetic risk factor. NAT2 has an important role in the metabolism of carcinogens and "slow acetylator" phenotype has been considered as risk factor for malignancy.

Our study included 51 women with diagnosis of CC and control group of 51 healthy women. Alleles determining fast acetylation (F1) or slow acetylation (S1, S2, S3) at NAT2 loci were detected by PCR-RFLPs method. The other risk factors were collected by questionnaire. It was calculated the frequency of gene polymorphisms, possible link with CC, frequency of other risk factors as well as combination of all estimated risk factors.

The most common genotypes in the CC and control group was F1S1and S2S3, respectively, both in the same percentage of 27.45%. Genotype S2S3 has been significantly frequent in CC women what is considered as a predisposition for disease. Protective role of F1 allele for CC was not established. Smoking, rare gynecological checks, as well as lack of knowledge of CC prevention are confirmed as important risk factors for disease.

The research indicated rolle of NAT2 polymorphism in CC and confirmed that regular gynecological check trough well organised screening is still best preventive method.

#### P06.048

##### The TaqIB Polymorphism of CETP Gene is Associated with Risk of Coronary Atery Disease (CAD) in Men in Russian Population

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CETP plays a central role in the metabolism of lipoproteins and might therefore alter the susceptibility to atherosclerosis. The TaqIB polymorphism of the *CETP* had been shown to be associated with its effect on lipid-transfer activity and on HDL cholesterol (HDL-CH) concentrations.

This study was undertaken to investigate genotype distribution and allele frequencies of *CETP* Taq B1 polymorphism in CAD patients of different age and sex and healthy individuals and to reveal its influence on HDL-CH concentration in these groups of patients.

594 patients were included in our study: 227 men who survived myocardial infarction (MI) under the age of 45 (group I), 95 men with MI after 60 years (group II) and 115 healthy men (group III); 73 angiographically diagnosed CAD women (group IV) and 84 women (age > 80 years) without CAD (group V), all originated from Saint-Petersburg, Russia. Genotypes were determined by PCR-RFLP, statistic:  $\chi^2$  tests, SPSS. In group I levels of total cholesterol, LDL-CH and triglycerides was significantly higher, and levels of HDL-CH lower in comparison with group II. We found that frequency of B1B2 genotype was significantly higher among patients from group I than among patients from group II and group III ( $p=0,02$  and  $p=0,04$  respectively; OR=2). There was no difference in B1 and B2 allele frequency in groups of CAD and healthy men. Our results allow to suggest that B1B2 genotype of *CETP* is a risk factor of CAD in men but not in women in analyzed groups.

**P06.049****Analysis of CFTR gene in Italian patients with idiopathic normocalciuric calcium nephrolithiasis**M. Gabaldo<sup>1</sup>, C. Bombieri<sup>1</sup>, A. Fabris<sup>2</sup>, A. Lupo<sup>2</sup>, P. F. Pignatti<sup>1</sup>, G. Gambaro<sup>2</sup>;<sup>1</sup>Section of Biology and Genetics, Dpt. of Mother and Child and of Biology-Genetics, University of Verona, Italy; <sup>2</sup>Division of Nephrology, Dpt. of Biomedical-Surgical Sciences, University Hospital of Verona, Italy.

Idiopathic calcium nephrolithiasis (ICN) is a multifactorial disease, whose pathogenesis involves a complex interaction of environmental and individual factors, possibly genetic. A growing body of evidence shows an association between Cystic Fibrosis (CF) and calcium nephrolithiasis. Of patients with CF, 3.0 to 6.3% are affected with nephrolithiasis, generally with calcium oxalate stones, a percentage greater than that of age-matched healthy controls (1-2%). CFTR mutations may be responsible for abnormal kidney development or may affect some steps of renal crystallization processes, thus favouring stone formation. Aim of this project was to investigate whether idiopathic normocalciuric calcium renal stone formers are associated with CFTR gene mutations or polymorphisms. A group of 44 Italian normocalciuric ICN patients and presenting no story reminding of CF were selected. Clinical and laboratory test to determine renal function were performed to exclude other causes of nephrolithiasis. The complete coding sequence and the intronic flanking regions of the CFTR gene were analysed by DGGE and sequencing. A total of 5/44(11%) patients had a mutation in the CFTR gene, all in heterozygosity (2 F508del, 1 D1152H, 1 R110H, 1 R75Q). This results is not statistically different from that found in a previous study on the general population (HumGenet, 106:172-178, 2000). The frequency of other common polymorphisms of the CFTR gene is also not different from the general population. These results show that the CFTR gene carrier status is not a risk factor for normocalciuric ICN.

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**P06.050****MTHFR gene polymorphisms as a risk factor for congenital heart disease in São Miguel island, Azores (Portugal)**R. Cabral<sup>1,2</sup>, F. Tejero<sup>1</sup>, L. de Fez<sup>1</sup>, P. R. Pacheco<sup>1,2</sup>, C. C. Branco<sup>1,2</sup>, C. P. Duarte<sup>3</sup>, R. Anjos<sup>4</sup>, L. Mota-Vieira<sup>1,2</sup>;

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Congenital heart defects (CHD) are among the most common birth defects worldwide, occurring in São Miguel Island with a prevalence of 9.16 per 1000 live births (Cymbron T et al. *Community Genet*, 9: 107-112, 2006). Several studies demonstrate that the intake, by the mothers, of periconceptional folic acid reduces the risk of congenital anomalies, including CHD. This fact led to the search of candidate genes involved in folate metabolic pathways. Methylenetetrahydrofolate reductase (MTHFR), a regulating enzyme of this metabolism, is responsible for the availability of active folate. Our main goal is to test the C677T and A1298C MTHFR polymorphisms as risk factor for CHD. We analyzed these two variants in a control population of 469 healthy blood donors from São Miguel and in the CHD group of 95 patients. For 677CC, 677CT and 677TT, we observed 28 (29.5%), 54 (56.8%), and 13 (13.7%) CHD cases, respectively. Similar proportions were found in the control individuals, that is, 162 (34.5%) were CC, 223 (47.6%) were CT and 84 (17.9%) were TT. Genotype frequencies of 1298AA, 1298AC and 1298CC were 56 (58.9%), 38 (40%) and 1 (1.1%) among cases and 264 (56.3%), 177 (37.7%) and 28 (6%) among control population, respectively. These values reveal no significant differences between both groups (odds ratio, 95% confidence interval). Because we have evidences of familial aggregation in CHD cases, this study is being concluded with the transmission/disequilibrium test in order to analyse the transmission distortion in the 89 nuclear families. Funded by Azorean Government (M1.2.1./I/003/2005). ritacabral@hdes.pt

**P06.051****Peripheral Gene Expression Profiling of CCK-4-Induced Panic in Healthy Subjects**K. Kallassalu<sup>1,2</sup>, E. Maron<sup>3,4</sup>, A. Tammiste<sup>1</sup>, R. Kolde<sup>5</sup>, I. Tõru<sup>4</sup>, V. Vasar<sup>4</sup>, J. Shiik<sup>6</sup>, A. Metspalu<sup>7,2</sup>;

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Panic attacks are anxiety-related phenomena defined as discrete periods of abruptly escalating intense fear or discomfort with multiple somatic and cognitive symptoms. Panic-induction or challenge tests in healthy subjects provide certain advantages in the investigation of genetic mechanisms of panic. Understanding why some, but not all, healthy subjects demonstrate panic response to a challenge test may advance knowledge about the pathogenesis of panic attacks and panic disorder.

In the present study we used the Illumina Human-6 v2 Beadchips for whole genome expression profiling in healthy subjects (n=31) participating in a cholecystokinin-tetrapeptide (CCK-4) challenge test. We aimed to explore the association between gene expression signatures, the occurrence of panic attacks, and the influence of the CCK-4 challenge on peripheral transcriptional activity.

The results showed that, after summarizing gene expression profiles before and after CCK-4 provocation, 16 genes were differently expressed between panickers and non-panickers ( $p<0.05$ ). Considering the higher susceptibility to panic attacks in females, we also performed separate analyses by gender. The transcriptional levels of 8 genes distinguished female panickers from female non-panickers. In males, 17 genes differed between panickers and non-panickers. Comparison of gene expression profiles in males and females revealed 5 genes expressed differently between genders. Gene expression profiling two hours post-CCK-4 challenge in all subjects, 78 genes showed significant changes in their transcriptional activity.

In summary, this study represents the first attempt to find associations between panic attacks and peripheral gene expression in humans using a challenge experiment design.

**P06.052****Genetic polymorphism in the matrix metalloproteinases genes MMP1, MMP9,MMP12 and risk of chronic lung disease in children**O. Tselousova<sup>1</sup>, G. Kortyna<sup>1</sup>, L. Akhmadishina<sup>1</sup>, T. Victorova<sup>1,2</sup>,

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The matrix metalloproteinases (MMPs) are proteolytic enzymes that play an essential role in lung tissue remodeling and repair during the inflammatory response. MMP1 (interstitial collagenase), MMP9 (gelatinase B) and MMP12 (macrophage elastase) may be important in the development of chronic lung disease. In our study, we investigated the role of common polymorphisms within several MMP gene promoters in genetic predisposition to chronic lung disease in children. DNA samples from 235 children with chronic respiratory diseases were analyzed. The control group consisted of 323 conditionally healthy persons, living in the Republic of Bashkortostan, Russia.

We have shown that the alleles and genotypes distribution of MMP12 gene A(-82)G polymorphism was significantly differed between patients with chronic lung disease and controls ( $\chi^2=5.45$ ,  $df=1$ ,  $P=0.02$  and  $\chi^2=5.85$ ,  $df=1$ ,  $P=0.016$ ). The AA genotype was identified as a risk factor for chronic lung disease in children ( $\chi^2=5.85$ ,  $df=1$ ,  $P=0.02$ ,  $P_{cor}=0.04$ ; OR=2.02, 95%CI 1.13-3.62). Genotype AG was more frequent in healthy children, and was identified as a protective genotype ( $\chi^2=5.85$ ,  $df=1$ ,  $P=0.02$ ,  $P_{cor}=0.04$ ; OR=0.50, 95%CI 0.28-0.89).

However, the genotype and allele frequencies of polymorphisms G(-1607)GG of MMP1 gene and C(-1562)T of MMP9 gene do not significantly differ in groups.

Our results showed that the polymorphisms in several MMP genes may play a significant role in the development of chronic lung disease in children.

**P06.053****Association study of microsatellite markers of five candidate loci in the cleft lip and palate patients of Lithuania**L. Ambrozaitytė<sup>1,2</sup>, A. Matulevičienė<sup>1,2</sup>, V. Kučinskas<sup>1,2</sup>;<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Centre for Medical Genetics, Vilnius University Hospital Santariskiu Klinikos, Vilnius, Lithuania.

The incidence of cleft lip and/or cleft palate (CL/P) in the population of Lithuania is 1 in 544 newborns; nonsyndromic CL/P cases are 74.1% part of it. Many different genes are considered as candidate loci for nonsyndromic CL/P responsible for this malformation. And the results by many study groups are still very inconsistent.

We have investigated five microsatellite markers D2S292 in TGFA gene, D14S61 in TGFB3 gene, D15S97 in GABRB3 gene, D17S1335 in RARA gene and BCL3 in BCL3 gene in 120 triads (child with non-syndromic CL+/-CP and both parents) - 102 triads with a child with nonsyndromic cleft lip with or without cleft palate (NS-CL+/-P) and 18 triads with a child with nonsyndromic cleft palate only (NS-CPO). Association analysis was performed by using transmission disequilibrium test (TDT).

Allele-wise and genotype-wise analysis gave no statistically significant results ( $p>0.05$ ). Nevertheless transmission disequilibrium analysis of every marker allele separately showed significant association between three out of investigated five microsatellite markers and NS-CL+/-CP: allele 6 (182 bp) of D2S292 marker ( $p=0.024$ ), allele 11 (206 bp) of D14S61 marker ( $p=0.025$ ) and allele 3 (128 bp) of BCL3 marker ( $p=0.015$ ).

These results suggest the contribution of TGFA, TGFB3 and BCL3 genes to nonsyndromic CL/P as well as indicate that these genes are probably not the causal genetic risk factors.

**P06.054****Intron region importance of *BCL3* gene in the nonsyndromic cleft lip and/or cleft palate**I. Prane<sup>1,2</sup>, B. Lace<sup>1</sup>, L. Piekuse<sup>1</sup>, J. Klovins<sup>2</sup>, B. Barkane<sup>3</sup>, I. Akota<sup>3</sup>, A. Krumina<sup>1</sup>;<sup>1</sup>Department of Molecular Biology and Genetics, Riga Stradiņš University, Riga, Latvia, <sup>2</sup>Latvian Biomedical Research and Study Center, Riga, Latvia, <sup>3</sup>Department of Oral and Maxillofacial Surgery, Institute of Stomatology, Riga Stradiņš University, Riga, Latvia.

**Background:** Orofacial clefts form as a result of interaction of environmental and genetic factors. Still up to now the exact mechanism of how the clefts form are not known. This is why it is so important to explore and investigate genes constituting to this process. It has been reported that *BCL3* (*B-cell leukemia/lymphoma-3*) on chromosome 19q13.1-q13.2, or a nearby gene may play a role in the etiology of nonsyndromic cleft lip and/or cleft palate (CLP/CP). There is possibility *BCL3* gene mutations increase affinity to transcriptional factors thus inhibited expression of genes, which are important in mesenchymal development process.

The aim of the study was to evaluate relevance of *BCL3* gene intron region in development of nonsyndromic orofacial clefts.

**Materials and methods:** Eight SNPs (rs7257231, rs1040117, rs8103315, rs2927457, rs11671085, rs1979377, rs2927456 and rs2306148) in the *BCL3* gene were analyzed with MALDI-TOFF technique for allelic association with the nonsyndromic CLP/CP in 75 trios (proband with both parents) from Latvia. Observed data were analyzed with transmission disequilibrium test (TDT).

**Results:** Three of eight SNPs (rs2927457, rs11671085 and rs2306148) were not polymorphic, five were found to be polymorphic - rs7257231, rs1040117, rs8103315, rs1979377 and rs2927456. TDT analysis revealed SNP rs10401176 ( $\chi^2=5.143$ ,  $P=0.023$ , df 1) in the CLP patient group and three SNPs rs10401176 ( $\chi^2=5.444$ ,  $P=0.0196$ , df 1), rs7257231 ( $\chi^2=4.455$ ,  $P=0.034$ , df 1), rs2927456 ( $\chi^2=4.000$ ,  $P=0.045$ , df 1) in the CP patient group showed statistically significant association.

**Conclusion:** Statistical analysis of obtained results showed *BCL3* gene intron 1 as an meaningful region for further studies.

**P06.055****Role of the C1236T (rs1128503) polymorphism of the MDR-1 Gene on Clopidogrel Responsiveness***E. Trabetti*<sup>1</sup>, M. Zanoni<sup>1</sup>, P. Prandini<sup>1</sup>, D. J. Angiolillo<sup>2</sup>, E. Bernardo<sup>3</sup>, A. Fernández-Ortiz<sup>3</sup>, C. Macaya<sup>3</sup>, T. A. Bass<sup>2</sup>, P. F. Pignatti<sup>1</sup>;<sup>1</sup>Department of Mother and Child and of Biology-Genetics, Section of Biology-Genetics, Verona, Italy, <sup>2</sup>Division of Cardiology, University of Florida College of Medicine-Shands Jacksonville, Jacksonville, FL, United States, <sup>3</sup>Cardiovascular Institute, San Carlos University Hospital, Madrid, Spain.

**Background:** Clopidogrel intestinal absorption and active metabolite formation are influenced by P-glycoprotein-mediated efflux. The functional activity of P-glycoprotein is under genetic control by the Multi Drug Resistance-1 (MDR-1) gene. If genetic variations of MDR-1 contribute to variability in clopidogrel response in patients with coronary artery disease remains poorly explored.

**Methods:** The C1236T (rs1128503) polymorphism of the MDR-1 gene was assessed in 62 patients. Patients were divided into 2 groups: carriers and non-carriers of the T allele. Platelet aggregation was performed before and 24 hours after clopidogrel administration. Standard (300mg; n=45) and high (600mg; n=17) loading dose regimens were used. All patients were on treatment with aspirin (100mg/day). Peak platelet aggregation was assessed by LTA using 6 µmol/L ADP stimuli. Post-treatment platelet reactivity and percentage inhibition of platelet aggregation (IPA) were determined.

**Results:** 71% and 29% of the study population were T and non-T allele carriers, respectively. At baseline, there were no differences in platelet aggregation between the two groups. At 24 hours there were no differences in post-treatment platelet reactivity between groups following a 300mg loading dose administration. However, following a 600mg loading dose administration, post-treatment platelet reactivity was significantly higher in T allele carriers ( $35\pm11\%$  vs  $16\pm3\%$ ;  $p=0.006$ ). Accordingly, there were no differences in IPA following a 300mg dose and IPA was significantly lower in T allele carriers following a 600mg dose ( $44\pm18\%$  vs  $73\pm5\%$ ,  $p=0.001$ ).

**Conclusions:** The C1236T polymorphism of the MDR-1 gene modulates clopidogrel responsiveness in the acute phase of treatment when using high loading dose regimens.

**P06.056****Association of cocaine dependence and neurotrophic factors: contribution of the Brain-Derived Neurotrophic Factor (BDNF) and the Neurotrophic Tyrosine Kinase Receptor 2 (NTRK2)***N. Fernandez-Castillo*<sup>1</sup>, M. Ribasés<sup>2</sup>, B. Gonzalvo<sup>2</sup>, M. Casas<sup>2,3</sup>, C. Roncero<sup>2,3</sup>, B. Cormand<sup>1,4</sup>;<sup>1</sup>Departament de Genètica, Facultat de Biologia. Universitat de Barcelona, Barcelona, Spain, <sup>2</sup>Servei de Psiquiatria, Hospital Universitari Vall d'Hebron, Barcelona, Barcelona, Spain, <sup>3</sup>Departament de Psiquiatria i Medicina Legal, Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>4</sup>CIBER Enfermedades Raras, Institut de Salud Carlos III, Barcelona, Spain.

Drug addiction is a complex psychiatric disorder that results from the interaction of different genetic and environmental factors. Tolerance, sensitization, craving and abstinence are common features in drug addiction, and may be related to neuronal processes such as plasticity and remodeling. Animal and pharmacological studies suggest that neurotrophins may play an important role in cocaine addiction through their effect on these processes. To evaluate the contribution of the Brain-Derived Neurotrophic Factor (BDNF) and its specific receptor Neurotrophic Tyrosine Kinase Receptor 2 (NTRK2) we genotyped two SNPs in the *BDNF* gene and three SNPs in the *NTRK2* gene in a Spanish sample of 91 cocaine dependent patients and 91 sex and age-matched healthy controls. The single-marker analysis showed association between cocaine dependence and the Val allele of the p.Val66Met *BDNF* polymorphism (rs6265;  $2p=0.014$ ; OR=2.14 (1.16-3.96)) and the c.2732T>C polymorphism of the *NTRK2* gene ( $2p=0.0062$ ). Additionally, the multiple-marker analysis supported an overrepresentation of the C/A/C haplotype (rs1187325/rs1047896/c.2732T>C) of the *NTRK2* gene in cocaine addicts ( $2p=0.005$ ; OR=7.19 (1.77-29.2)). Our results, although preliminary, point out that the *BDNF* and *NTRK2* genes may contribute to the predisposition to cocaine addiction and suggest a potential participation of neurotrophic factors in drug dependence.

**P06.057****Clinical and immunohistochemical pre-screening and RNA sequencing increase mutation detection rate for the Collagen VI genes**

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Ullrich Congenital Muscular Dystrophy (UCMD) and the milder Bethlem Myopathy (BM) are caused by mutations in COL6A1, COL6A2 and COL6A3 which collectively are comprised of 106 coding exons. Neither phenotypic nor immunohistochemical analyses are able to pinpoint which of these genes to target for mutation screening.

We have established a diagnostic mutation screening service for these three genes based on sequencing cDNA derived from fibroblast cultures. This requires only 26 overlapping cDNA fragments, compared with over 100 fragments needed to cover the genes using genomic sequencing.

Mutations have been detected in 14 out of an initial cohort of 16 patients; this 87.5% detection rate compares favourably with the 62% detection reported in Lampe et al. This higher rate of detection can be partly attributed to the sequential use of clinical and immunohistochemical screening prior to molecular analysis and partly to the RNA-based approach. In particular a change ten bases from the start of the exon in COL6A2 (c.1117-10A>G) is likely to be responsible for the splicing out of exon 13 in one patient, and in a second patient an absence of exon 10 at the cDNA level of the same gene has as yet no confirmed mechanism at the DNA level.

Surprisingly eight patients with a UCMD phenotype, previously thought of as a recessively-inherited form, have a single mutation; this reinforces current thinking that the two disorders represent either end of a phenotypic spectrum.

Lampe, A. K. et al; J Med Genet 2005;42:108-120

**P06.058****A computational test for biological relatedness in genetic association studies using probabilistically inferred haplotypes**

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An association between gene and disease may be incorrectly estimated if the allele frequencies differ among cases and controls depending on inbreeding or unrecognized population stratification.

A program (<http://medgen.univr.it/jenoware/>) was developed to compute the probability of genetic relatedness in pairs of individuals using a likelihood ratio test.

Using loci that are in LD decreases the accuracy of parentage assignments. Groups of SNPs in linkage disequilibrium (LD) were simulated to verify the effects of linkage on relatedness assignment. The probability of genetic relatedness was computed using the single SNPs and treating the SNPs as composite markers with different  $r^2$  threshold values. Haplotypes were probabilistically inferred using the PHASE and Gerbil programs. False positive rate and power were assessed by simulation in unrelated individuals and in pedigrees.

As an example of results, in order to estimate the support for II degree relatedness with power 80%, and false positive rate 5%, the following was needed: 100 SNPs with no linkage; 275 SNPs having  $r^2=0.4$ ; 20 probabilistically inferred haplotypes (100 SNPs having  $r^2=0.4$ ); 40 probabilistically inferred haplotypes (200 SNPs having  $r^2=0.8$ ).

In conclusion, if LD blocks are examined, the biological relatedness can be computed with a limited number of markers increasing test accuracy with probabilistically inferred haplotypes.

**P06.059****A new gene for X-linked Congenital Ataxia maps to Xq25-q27.1**

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Pediatrics, Justus Liebig Universität, Giessen, Germany, <sup>6</sup>Department of Pediatrics, Medical Genetics, Human Genetics, Pathology, Obstetrics and Gynaecology, University of Utah, Salt Lake City, UT, United States.

We observed a large American family of Norwegian descent with X-linked nonprogressive congenital ataxia (XCA) and normal cognitive development in six affected males over three generations. Neuroimaging showed global cerebellar atrophy without evidence of supratentorial anomalies. Linkage analysis resulted in a maximum LOD score  $Z=3.44$  for marker DXS1192 at  $\theta=0.0$  with flanking markers DXS1047 and DXS1227 defining a region of 12cM in Xq25-q27.1. The clinical and neuroradiological findings in the present family are very similar to those described in two other reported X-linked families however, the newly identified locus does not overlap with the one defined previously in Xp11.21-Xq24, indicating that there are at least two genes responsible for this rare form of X-linked congenital cerebellar ataxia with normal intelligence.

**P06.060****Autosomal dominant left atrial isomerism with suggestive linkage to chromosome 9q**

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**Background**

Left isomerism is a laterality disorder, characterized by bilateral left-sidedness including cardiovascular malformations, bilateral bilobed lungs and polysplenia. Autosomal dominant laterality disorders are infrequent and have only been reported in a few families. In this study, we analyzed a large three generation family with cardiac left isomerism.

**Methods and results**

We obtained phenotypic information, including physical examination, electrocardiography, echocardiography and blood samples of 22 family members. Thirteen individuals had a cardiac anomaly, with considerable variation between patients. Cardiac anomalies considered to be part of the left isomerism spectrum were, among others, bilateral left atrial appendages, septal defects, persistent left superior caval vein and specific electrocardiographic disturbances compatible with absence of the sinoatrial node. Other heart defects were present as well. The condition was inherited in an autosomal dominant pattern. Subsequent genome wide linkage analysis demonstrated linkage to a single locus on chromosome 9q shared by all affected individuals, with a multipoint maximum LOD score of 2.20 at marker D9S283. The shared locus was delineated by markers D9S167 and D9S1677, was 26 Mb in size and contained 218 genes. Sequence analysis of three candidate genes in this region (Inversin, TGFBR1 and IPPK) revealed no mutations.

**Conclusions**

The mapping of a suggestive locus for this autosomal dominant laterality disorder on chromosome 9q represents an important step toward the discovery of genes implied in laterality disorders. Further sequencing and investigation of genomic duplications/deletions of candidate genes will be the next steps in the identification of the susceptibility gene.

**P06.061****Implication of HOXB9 and COL1A1 genes in congenital hip dislocation : a case-control association study in Brittany (Western-France)**

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Congenital dislocation of the hip (CDH) is a complex disease which presents a mechanical component due to the pregnancy and delivery conditions and a genetic component linked to the ethnical predispositions and the familial aggregation. A case-control association study was set up in the area of Finistère (western Brittany, France) where CDH is frequent in order to study the implication of two candidate genes,

HOXB9 involved in the embryonic development of members and COL1A1 implied in the constitution of cartilaginous tissue. TagSNPs were selected using Haploview and Tagger softwares ( $r^2=0.8$ , MAF=0.05) and genotyped using SNaPshot method (HOXB9: n=3 and COL1A1: n=12). All SNPs were in Hardy-Weinberg equilibrium. Associations were tested using logistic regression analysis. Our cohort included 239 CDH patients and controls and CDH affected 91.2% of women and was bilateral in 60.8% of cases. Breech presentation was significantly associated with CDH (OR=10.10 [2.96-34.46], p<0.0001) and hyperlaxity was also more frequent in cases (OR=6.74 [2.31-19.71], p<0.0001). On genetic aspects, no significant association was observed between CDH and the markers of HOXB9 gene. The analysis of COL1A1 gene revealed significant association for two markers: rs1107946 (genotype AA+AC vs CC: p=0.0379) and rs2857396 (genotype TT+TC vs CC: p=0.0426). These findings were not confirmed after Bonferroni correction. An interaction between primiparas and rs1107946 was observed (p=0.0491). A replication study (TDT) and a haplotype analysis are still in process and will better explain the role of this two genes in CDH. This work was supported by a grant from PHRC and PRIR.

#### P06.062

#### Endothelial nitric oxide synthase polymorphism in children with connective tissue dysplasia in Saint-Petersburg

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Connective tissue dysplasia (CTD) is a group of heterogenous diseases with hereditary and inborn collagen synthesis disturbance. These patients have risk of vascular disorders. Previous studies have suggested an influence of gene endothelial nitric oxide synthase (eNOS) on vascular disorders (VD) in adults and children.

The aim of our study was to detect distribution polymorphic eNOS genotypes and alleles in CTD children and healthy children in Saint-Petersburg.

101 CTD children (38 girls, 63 boys) and 146 healthy Saint-Petersburg children (70 girls and 76 boys) were included in our study. The mean age of CTD patients was 12,71 ±4,88 years. We detected 4a/4b gene polymorphism eNOS (4a with 27-bp repeats, 393 bp total; 4b with five tandem repeats, 420 bp total), eNOS gene promoter T-786C SNP and glu298-to-asp polymorphism eNOS in these children by PCR.

The distribution of 4a/4b polymorphism has revealed significant differences (p=0,025) in genotypes frequency between CTD and healthy boys.

The distribution of eNOS T-786C SNP polymorphism has revealed significant differences (p=0,015) in genotypes frequency between CTD and healthy girls.

The distribution of glu298-to-asp polymorphism eNOS has revealed significant differences (p=0,015) in genotypes frequency between all groups. (Dates in table)

Conclusion: allele C of eNOS T-786C and allele T of glu298-to-asp eNOS, associated with vascular disturbances were frequent in children with CTD.

	4a/4b		T-786C		Glu298Asp	
	CTD	Controls	CTD	Controls	CTD	Controls
Girls	n=38 n=69		n=37 n=70		n=38 n=70	
Genotype, p value	aa: 0,0 0,07		TT: 0,16 0,44		GG: 0,29 0,54	
Alleles, p value	ab: 0,26 0,29		TC: 0,7 0,47		TG: 0,37 0,37	
	bb: 0,74 0,64		CC: 0,13 0,09		TT: 0,34 0,09	
	0,2		0,015		0,002	
	0,17		0,026		0,000	
Boys	n=63 n=73		n=62 n=75		n=63 n=76	
Genotype, p value	aa: 0,02 0,04		TT: 0,31 0,32		GG: 0,24 0,51	
Alleles, p value	ab: 0,19 0,34		TC: 0,53 0,49		TG: 0,49 0,41	
	bb: 0,79 0,61		CC: 0,16 0,19		TT: 0,27 0,08	
	0,07		0,88		0,000	
	0,038		0,98		0,000	
Total	n=101 n=142		n=99 n=145		n=101 n=146	
Genotype, p value	aa: 0,01 0,06		TT: 0,25 0,38		GG: 0,26 0,53	
Alleles, p value	ab: 0,22 0,31		TC: 0,6 0,48		TG: 0,44 0,39	
	bb: 0,77 0,63		CC: 0,15 0,14		TT: 0,3 0,08	
	0,025		0,11		0,000	
	0,009		0,15		0,000	

#### P06.063

#### Association of Copy Number Variation at 22q11.23 with Schizophrenia

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Copy number variation (CNV) is likely to be a substantial source of human genetic diversity, influencing the variable susceptibility to multifactorial disorders. However, the relatively high cost and false positive rate of genome wide methodologies have still precluded large scale association studies in patients and controls. Schizophrenia is a complex illness thought to be caused by a number of genetic and environmental effects, few of which have been clearly defined. We have used a multiplex-ligation-PCR-amplification based method to target previously reported and putatively relevant gene-containing CNV regions. A total of 122 genes were studied in 451 schizophrenic patients and 304 matched controls for association studies. Most genotyped CNVs (84%) showed very low (<1%) population frequency, while only few were common variants whose frequency did not differ between groups. A few rare variants were only present in patients suggesting a possible pathogenic involvement. We found two patients with amplification of two consecutive probes in 22q11.23 that were further characterized. Both patients showed 1.2 Mb overlapping duplications spanning 24 genes, being *de novo* in the case for whom the parents were available. A specific assay targeting this complex 22q11.23 region revealed two additional CNVs significantly associated with increased risk for schizophrenia, the duplication of the *DDT* gene and the positive genotypes of the *GSTT2* gene (Fisher, p<0.01). Our data provide complementary evidence for chromosome 22q11.23 as a susceptibility locus for schizophrenia, and suggest that CNV within this region may influence risk.

#### P06.064

#### A new type of hereditary corneal recurrent erosions with late subepithelial fibrosis - Dystrophia Helsingiensis

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We describe the phenotype of a large family with an autosomal dominant inherited corneal dystrophy characterized by recurrent corneal erosions and late subepithelial fibrosis. The disorder was not linked to any known gene causing autosomal dominant corneal dystrophies with a clinical resemblance. The pedigree consisted of 342 individuals of whom 85 were affected by the disorder. The affected family members had erosive symptoms that usually lasted from 1 to 10 days. By the age of 7 almost all of the affected individuals suffered from recurrent corneal erosions. The attacks generally declined in frequency and intensity, but all individuals had developed subepithelial fibrosis by the age of 40. The fibrosis generally started in the midperiphery and were followed in some family members by the development of gelatinous masses or keloid-like formations. Nevertheless, only a marginal reduction of visual acuity was seen, and thus no one received corneal grafts. The affected individuals did not share haplotypes for genetic microsatellite markers surrounding the known genes (*COL8A2*, *TGFBI*, *GSN*, *KRT3*, *DCN*, *KRT12*) causing autosomal dominant corneal dystrophies with a similar phenotype and thus we conclude that the family had a previously not described corneal dystrophy. A genome wide scan is now being performed to locate the disease causing gene.

**P06.065****Frequency, significance and association of ACE I/D and MTHFR C677T gene polymorphisms in Turkish patients with early onset coronary artery disease**

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**Introduction:** Coronary artery disease (CAD) is a polygenic disease. A number of polymorphisms, including the insertion (I) or deletion (D) of a 287 bp Alu repeat sequence polymorphism of angiotensin I converting enzyme (ACE) gene and a point mutation (C677T) of 5,10-methylene-tetrahydrofolate reductase (MTHFR) gene have been implicated in the pathogenesis of CAD. We investigated ACE and MTHFR genotypes in patients with angiographically documented CAD in Turkish population.

**Methods:** 184 patients (aged between 23-72, mean  $45 \pm 7.5$  years) were included in the study. DNAs from 89 patients with angiographically proven CAD (age<45 for male, <55 for female) and 95 controls without CAD were amplified.

**Results:** The ACE genotype DD was present in 32.6% of patients with CAD as compared to 37.9% in the controls ( $p = 0.45$ ). Genotype frequencies in all groups for II, ID and DD were: 21.7%, 42.9%, 35.4% respectively. The overall frequency of the MTHFR genotypes were %70.7 (CC), %22.8 (CT) and %6.5 (TT). The MTHFR genotype TT was present in 4.5% of patients with CAD as compared to 8.4% in controls ( $p = 0.38$ ). Subgroup analysis showed no significant differences between CAD and CAD free subjects. Also, the ACE DD and MTHFR TT genotypes were not found more common in patients having multi-vessel CAD when compared to single-vessel disease.

**Conclusion:** I/D polymorphism of ACE gene and C677T polymorphism of MTHFR gene are not independent risk factors for CAD in the studied Turkish population. Paraoxonase, PAI1 and e-NOS polymorphism detections are in progress for these patients.

**P06.066****Quantitative Trait Mapping of Signaling Pathways in Human Primary Cells**

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Thousands of mammalian cell types each express a unique complement of receptors, ion channels, and enzymes. Nevertheless, the signaling pathways that transduce outside signals to the transcriptional machinery in these cells remain exquisitely conserved. Their downstream effector molecules are generally regulated by phosphorylation, and bind to conserved elements in hundreds of promoters to control a variety of processes. Such elements include CRE boxes, bound by CREB to mediate adenyl cyclase-dependent transcription; SRE boxes, bound by SRF to mediate JNK- and ERK-family MAP kinase-dependent transcription; and E boxes, which are bound by CLOCK:BMAL1 heterodimers to mediate circadian transcription. We are generating a series of viral reporters for these pathways that are capable of infecting human primary cell cultures. The signals from these reporters can be used as quantitative traits in association and linkage studies to identify human modifier loci for these pathways. For example, using an E box reporter, we have been able to explore the molecular properties of the circadian clock in people of early and late chronotype ("larks" and "owls"). We hope to use these cellular assays to map the genes that determine human differences in a variety of difficult-to-access behavioral phenotypes that might include daily behavior, mood, and memory.

**P06.067****CARD15 gene polymorphisms in Serbian patients with Crohn's disease: Genotype-phenotype correlation**

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Caspase activation and recruitment domain 15 (CARD15) has been identified as the pivotal gene associated with Crohn's disease (CD). Several studies have shown that CARD15 polymorphisms increase susceptibility for CD and are associated with ileal location and fibrostenotic behaviour of the disease. The aim of this study was to evaluate the prevalence of CARD15 polymorphisms and their phenotypic correlation in Serbian patients with CD. 131 patients with well defined CD and 88 healthy controls were genotyped for three common polymorphisms (R702W, G908R and Leu1007insC) by PCR-RFLP. CARD15 variant was found in 46/131 (35.11%) patients with CD and 13/88 (14.77%) of healthy controls ( $p=0.001$ ). The frameshift polymorphism (L1007fs) showed a significant association with CD (15.27%) compared to control group (0.00%) ( $p<0.0001$ ). The frequency of R702W was remarkably higher in control group (14.77%) and almost equal to occurrence of this particular polymorphism in patients with CD (20.63%). Polymorphism G908R was observed significantly more frequent in patients with CD (5.3%) than in control group (0.00%) ( $p=0.043$ ). Univariate analysis established that carriers of CARD15 common polymorphisms had a significantly higher risk of isolated ileal location ( $p=0.042$ ; OR 1.650; 95% CI 1.04-2.60), fibrostenotic behaviour ( $p<0.0001$ ; OR 3.143; 95% CI 1.58-6.25) and surgical resection ( $p=0.036$ , OR 2.2; CI 1.046-4.626). This study confirms that carriers of CARD15 polymorphisms have an increased risk of CD. The data show that polymorphisms are associated with ileal, fibrostenosing disease and a higher risk of surgery.

**P06.068****Prevalence of IGR2230a\_1 genotypes in Hungarian Crohn's disease and ulcerative colitis patients**

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Two main clinical presentations of inflammatory bowel disease are Crohn's disease and ulcerative colitis. IBD is caused by a combination of genetic, environmental and immunological factors. Association has been repeatedly demonstrated between IBD and the IBD5 locus on chromosome 5q31 (Silverberg et al; Eur J Hum Genet. 2007 Mar;15(3):328-35.). The aim of the study was to examine the prevalence of the IGR2230a\_1 intronic nucleotide polymorphism of the SL-C22A5 gene (coding for the OCTN2 carnitine transporter protein) in Hungarian IBD patients and controls. We examined 200 patients with CD (97 males, 103 females, mean age  $39.4 \pm 14.6$  years) and 246 patients with UC (108 males, 138 females, mean age  $44.0 \pm 15.1$  years). A group of 187 carefully selected, clinically healthy subjects (106 males, 81 females, mean age  $37.7 \pm 10.7$  years) were collected for the study. For genotyping we used PCR/RFLP methods. The prevalence of the mutant allele A of IGR2230a\_1 was 48.5 % in the CD and 47.1 % in the UC patients, while it was 44.6 % in the controls. The frequency of the homozygous AA genotype was similar in all three groups, and the wild type GG genotype was the most frequent in the controls (31.6 %), less in the UC cohort (28.4 %) and the least among the CD patients (23.5 %). None of the reasonable comparisons of genotypes resulted in statistically significant difference between patients and controls.

**P06.069****Interaction of IL23R 3'-UTR and ATG16L1 T300A in Hungarian Crohn's disease patients**

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Recently, we found significant association between Crohn's disease (CD) and rs10889677 (3'-UTR C2370A) of the interleukin-23 receptor (IL23R) gene in Hungarian patients (Faragó et al.; Ann Rheum

Dis. 2008 Feb; 67(2):248-50. Epub 2007 Jul 2.). The aim of this study was to investigate the statistical interaction of this CD susceptibility factor and rs2241880 (A1338G, T300A) of the autophagy-related 16-like 1 (ATG16L1) gene (Hampe et al.; Nat Genet. 2007; 39:207). 198 Hungarian patients with CD and 225 healthy controls were genotyped by PCR-RFLP methods. We found significantly higher frequency for the ATG16L1 G allele and GG genotype in the CD cohort compared to controls ( $p=0.008$ , OR=1.454, 95% CI: 1.106-1.910;  $p=0.0001$ , OR=3.460, 95% CI: 2.086-5.740). The frequencies and odds ratios of rs10889677 genotypes were stratified by rs2241880 genotypes. The ATG16L1 AG genotype significantly increased the risk for CD on the background of IL23R 3'-UTR CA and AA ( $p_{CA}=0.043$ , OR=2.522, 95% CI: 1.043-6.097;  $p_{AA}=0.013$ , OR=4.550, 95% CI: 1.464-14.145). The ATG16L1 GG genotype significantly increased the risk for CD with the susceptibility alleles of IL23R 3'-UTR ( $p_{CA}=0.004$ , OR=4.000, 95% CI: 1.553-10.306;  $p_{AA}=0.0001$ , OR=32.50, 95% CI: 3.59-294.216). The significantly highest relative odds ratios for rs2241880 were detected on the background of the IL23R AA genotype, suggesting the risk alleles of these two disease-associated loci have an additive effect.

#### P06.070

#### **IL1RL1-IL18R1-IL18RAP-SLC9A4 and CARD9 loci are susceptibility factors for both Crohn's disease and ulcerative colitis**

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The two main phenotypes of inflammatory bowel disease (IBD) - Crohn's disease (CD) and ulcerative colitis (UC) - are chronic intestinal inflammatory disorders with a complex genetic background.

We performed a functional candidate gene analysis within the innate immune pathway in IBD using a three-stage design. In phase I, 354 SNPs from 85 innate immunity genes were typed in a cohort of 520 Dutch IBD patients (284 CD, 236 UC) and 808 controls. In phase II, 9 SNPs showing association at  $p<0.006$  in phase I were replicated in a second independent cohort of 545 Dutch IBD patients (326 CD, 219 UC) and 360 controls. In phase III, 3 SNPs with  $p<0.01$  in the combined phase I and phase II analysis were genotyped in an additional cohort of 786 Dutch IBD samples (452 CD, 334 UC) and 768 independent controls. Joint analysis of 1,851 IBD patients (1062 CD, 789 UC) and 1936 controls demonstrated strong association to the *IL18RAP* gene for both CD and UC ( $p_{IBD}=1.9E-8$ , OR 1.35). Association in CD is independently supported by the Crohn's disease dataset of the Wellcome Trust Case Control Consortium. In addition, an association of the *CARD9* variant to CD and UC was observed ( $p_{IBD}=3.25E-5$ , OR 1.21). Both genes are located in extended haplotype blocks on 2q11-2q12 and 9q34.3, respectively. Our results indicate two novel IBD loci and further support the importance of the innate immune system in the predisposition to both CD and UC.

#### P06.071

#### **Prevalence of IGR2198a\_1 and IGR2096a\_1 genetic variants in Hungarian patients with Crohn's disease and ulcerative colitis**

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Two major forms of inflammatory bowel disease (IBD) are Crohn's disease (CD) and ulcerative colitis (UC). The development of these diseases is caused by both environmental factors and complex genetic predisposition. Genetic association was found between IBD and IBD5 on chromosome on 5q31. Our goal was to analyse the possible influence of two variants in this region IGR2096a\_1 (rs12521868) and IGR2198a\_1 (rs11739135) in Hungarian IBD patients. PCR/RFLP methods were used for detecting the various genotypes. We examined 226 patients with CD (105 males, 121 females, age:  $40.5 \pm 14.5$  mean

$\pm$  S.D.), and 260 patients with UC (112 males, 148 females, mean age:  $48.7 \pm 15.9$ ). 156 carefully selected clinically healthy, age-, sex-, weight- and height-matched control subjects (85 males, 71 females, mean age:  $45.9 \pm 10.2$ ) were collected for the study. The IGR2198a\_1 C allele and the IGR2096a\_1 T allele was present in a significantly higher frequency in both CD (45.1%,  $p=0.004$ , OR 1.554, 95% CI 1.154-2.093; 46.5%,  $p=0.01$ , OR 1.639, 95% CI 1.217-2.207) and UC (42.3%,  $p=0.028$ , OR 1.385, 95% CI 1.035-1.853; 42.1%,  $p=0.028$ , OR 1.394, 95% CI 1.042-1.866) cohorts compared to controls (34.6% and 33.6% respectively). The IGR2198a\_1 CC and IGR2096a\_1 TT genotype frequencies were also increased, comparing the CD group to the controls (18.6%,  $p=0.043$ , OR 1.865, 95% CI 1.019-3.415; 22.1%,  $p=0.009$ , OR 2.178, 95% CI 1.216-3.902). We concluded that IGR2096a\_1 and IGR2198a\_1 SNPs mean susceptibility to both CD and UC in the Hungarian population.

#### P06.072

#### **49A/G and CT60 polymorphisms of the CTLA-4 gene associated with Graves' disease but not with Hashimoto's thyroiditis and postpartum thyroiditis**

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Single nucleotide polymorphisms in the CTLA-4 gene have been suggested as genetic factors in susceptibility to autoimmune thyroid disease (AITD). In our case-control study Graves' disease (GD), Hashimoto's thyroiditis (HT), postpartum thyroiditis (PPT) patients and control subjects from the Slovenian population were genotyped for two A/G single nucleotide polymorphisms (49A/G and CT60) of the CTLA-4 gene. The 49A/G polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (RFLP) analysis using BseXI. CT60 was genotyped using a real-time polymerase chain reaction (RT-PCR) and results were analyzed by the  $\chi^2$  test.

We found no significant difference, for either polymorphism, between the frequencies of AA and AG genotypes in control versus AITD patients. However, the frequency of the GG genotype was significantly higher in AITD patients versus controls for 49A/G ( $p<0.005$ ; 102 controls, 301 AITD patients), and for CT60 polymorphism ( $p=0.0096$ ; 150 controls, 345 AITD patients). Comparing frequencies of the GG genotype in GD, HT and PPT with controls, a significant association of 49A/G and CT60 polymorphism was shown for GD whereas association of these polymorphisms with HT and PPT could not be confirmed. In 123 GD patients the frequency of GG genotype (13.8 %) was significantly higher ( $p=0.0452$ ) versus 102 controls (4.9 %). The frequency of the GG genotype at CT60 was significantly higher in the group of 150 GD patients (42%;  $p=0.0113$ ), versus 150 control subjects (27.3 %). We conclude that 49A/G and CT60 polymorphism contributes to predisposition to GD, but not for HT and PPT.

#### P06.073

#### **Sequencing the steroid-21-hydroxylase gene and linkage analysis of the detected polymorphisms**

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The steroid-21-hydroxylase enzyme (CYP21) plays a crucial role in the synthesis of steroid hormones. Its gene is located in the MHCIII region in a strongly linked modular structure. This region referred to as RCCX contains four genes: RP, complement C4A or C4B, CYP21 and TNX. The number of RCCX modules may vary from 1 to 3 on a chromosome. Our previous results showed that individuals with 0 or 1 C4B gene (i.e. C4B\*Q0) have higher risk for myocardial infarction and stroke; moreover they were selected out from the healthy elderly population. The aim of the present study was to reveal the possible molecular genetic background of these observations. We assumed that C4B\*Q0 carrier state may result in impaired function of the neighboring CYP21 gene, associated with inadequate mobilization of steroid hormones during stress in critical situations. The CYP21 gene was sequenced in 96 healthy individuals, in whom the copy number of C4 genes and the genotype of three SNPs located in the MHCIII region

(RAGE -429T/C, HSP70-2 1267A/G and TNF $\alpha$  -308G/A) were also determined. We identified 53 polymorphisms in the CYP21 gene; ten SNPs were previously not reported. C4B\*Q0 was found to significantly linked to two intronic SNPs (1106C/A and 1113T/C) of CYP21. Analysis of 34 families confirmed that the haplotype block involving the variant form of the SNPs has no C4B gene. These results indicate that the SNPs may contribute to the higher morbidity and mortality rate of C4B\*Q0 carriers, presumably by influencing the expression of the CYP21 protein.

#### P06.074

#### Bisphosphonate related Osteonecrosis of the jaw is associated with polymorphisms of the Cytochrome P450 2C8 in Multiple myeloma: a genome wide single nucleotide polymorphism analysis

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Osteonecrosis of the jaws (ONJ) is an adverse side effect of bisphosphonate (BP) therapy. Patients with multiple myeloma (MM) usually receive BPs for the treatment of bone destruction. ONJ could be caused by a combination of environmental and genetic risk factors. Our aim was to assess the role of genetics in ONJ development. We performed a genome wide association study using 500.568 SNPs in two series of MM patients included in the same therapeutic protocol receiving the same BP therapy: 22 cases (MM with ONJ) and 65 matched controls (MM without ONJ).

Clinical and biological characteristics, response to treatment and survival rates were similar in both subsets of patients. Regarding the polymorphisms, we identified four SNPs (rs17110453, rs1934951, rs1934980 and rs1341162) mapped within the Cytochrome P450-2C gene (CYP2C8) with a singular distribution among cases and controls. Rs1934980, rs1341162 and rs17110453 showed a significant correlation with ONJ ( $p=4.231e^{-6}$ ,  $p=6.22e^{-6}$  and  $p=2.15e^{-5}$  respectively), although the association was not significant after Bonferroni correction. The SNP rs1934951 kept its statistical significant association with ONJ ( $P\text{-value}=1.07e^{-6}$ ,  $P\text{ corrected value}=0.02$ ). Genotyping results displayed an overrepresentation of the T allele in cases vs. controls (0.475 vs. 0.125). Thus, individuals homozygous for the risk allele had a likelihood of ONJ increased by 12.75 (95% confidence interval 3.7 to 43.5).

Our data suggest that the rs1934951 polymorphism may play a role as a risk factor for developing ONJ in MM patients receiving BPs therapy.

#### P06.075

#### CYP2D6 polymorphism in patients with Parkinson's disease

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Aim: CYP2D6 is an enzyme of cytochrome P-450 system that metabolizes some of endogenous substances in central nervous system, including metabolism of dopamine and drugs used for Parkinson's disease (PD) treatment. Decreased metabolic capability of this enzyme could be associated with increased risk of morbidity and of higher risk for side effects of antiparkinsonian medication. The aim of this study was determination of the incidence and comparison of non-functional alleles with the intention of detecting increased risk for PD in individuals with damaged function of enzyme CYP2D6.

Materials and methods: Multiplex allele-specific polymerase chain reaction (PCR) the incidence of non-functional alleles CYP2D6\*3, \*4, \*6, \*7, and \*8 was determined in healthy volunteers ( $n=145$ ) and in patients with PD ( $n=41$ ).

Results: In a group of healthy volunteers the incidence of CYP2D6 alleles was: CYP2D6\*3=1.4%, CYP2D6\*4=11.0%, CYP2D6\*6=1.0%, CYP2D6-wt=86.6%.

In a group of PD patients the incidence of CYP2D6 alleles was: CYP2D6\*3=1.2%, CYP2D6\*4=20.7%, CYP2D6\*6=1.2% and CYP2D6-wt=76.8%. Statistically significant difference was found only for allele CYP2D6\*4 (relative risk=2.10; 95% CI: 1.113-3.994).

The relation of genotype distribution was \*3/wt 2.8% and 2.4%; \*4/wt 18.6% and 26.8%; \*4/\*4 1.4% and 7.3%; \*6/wt 1.4% and 2.4%; \*4/\*6

0.7% and 0.0%; wt/wt 75.2% and 61.0% in healthy volunteers and PD patients, respectively). There was no statistically significant difference between these distributions.

Discussion: Results of this study indicate that the allele CYP2D6\*4 could be considered as a weak risk factor for PD, but similar study should be carried out on larger sample group.

#### P06.076

#### Contribution of gene sequence variant CYP3A4\*1B of the hepatic cytochrome P450 3A4 enzyme to variability in individual response to clopidogrel

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Clopidogrel is an inactive pro-drug that requires oxidation by hepatic cytochrome CYP3A4 to generate active metabolite. Because genetic variations are the major determinant of heterogeneity in metabolic activity of enzyme, we hypothesize that polymorphism of CYP3A4 gene may influence platelet aggregation in patients treated with clopidogrel. We examined platelet aggregation in 100 patients with acute coronary syndrome treated by clopidogrel 75mg/day according to variant CYP3A4\*1B. Aggregation was induced by ADP 20mM and measured in two points - before and on day 7 after clopidogrel treatment as level of light transmission (LT,%) determined by optical aggregometry. For detection of the CYP3A4\*1B the PCR and original endonuclease digestion with PstI was used and results in patients were compared with 83 healthy persons. Our study showed that the frequencies of CYP3A4\*1B genotypes were 89%, 10% and 1% in patients and 90.5%, 8.5% and 1% in controls for wild, heterozygous and homozygous genotypes, respectively, and these frequencies didn't differ in two groups. The LT was significantly lower in patients in second measuring point compared to first point -  $20.59 \pm 1.58\%$  and  $34.03 \pm 1.96\%$ , respectively ( $p<0.0001$ ). The wild genotype CYP3A4\*1B was associated with significant reductions in platelet aggregation after clopidogrel treatment - LT was  $33.41 \pm 2.06\%$  versus  $19.81 \pm 1.65\%$  in first and second measuring point respectively ( $p<0.0001$ ). In carriers of CYP3A4\*1B mutant allele the reduction of platelet aggregation was poorly effective and LT was  $37.96 \pm 6.26\%$  versus  $25.59 \pm 4.95\%$  in first and second measuring point respectively ( $p>0.1$ ). In conclusion, the CYP3A4\*1B polymorphism may contribute to poorly clopidogrel effect on platelet aggregation.

#### P06.077

#### Association of CYPs, GSTs, CAT, GPX1, SODs genes polymorphisms with chronic lung disease in children

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To assess the role that polymorphisms of cytochrome P450 and antioxidant-related genes play in genetic predisposition to severe chronic lung disease of children, the allele and genotype distributions of CYP1A1(2455A/G, 3801T/C), CYP1A2 (-2464T/deT, -163C/A), CYP2E1 (-1053C/T), GSTM1 (Del), GSTT1 (Del), GSTP1 (Ile105Val, Ala114Val), CAT (-262 C/T, 1167 C/T), GPX1 (Pro197Leu), SOD1 (3intron+35 A/C), SOD3 (Arg213Gly) genes were studied in children with chronic lung disease (N=188) and healthy children (N=300), living in the Republic of Bashkortostan in environment with air pollutions.

Genotypes of cytochrome P450 and antioxidant genes that associated with susceptibility to chronic lung disease of children were determined. The frequency of 1A2C genotype of CYP1A1 gene was significantly higher in cases of chronic lung disease patients than in healthy control group (15.43% vs 8.67%;  $X^2=4.626$ ,  $df=1$ ,  $P=0.0318$ ,  $Pcor=0.0636$ ; OR=1.922 95%CI 1.055-3.507). The patients with chronic lung disease showed significantly elevated frequencies of the GSTT1 gene deletion (38.83% vs 21.33% in control;  $X^2=16.66$ ,  $df=1$ ,  $P=0.0006$ ; OR=2.305 95% CI 1.512-3.515). The distribution of the GSTP1 gene alleles was significantly differed from that of chronic lung disease patients and controls, there was a higher frequency of the Ile/Ile genotype in the patients than in healthy group (76.06% vs 60.0%;  $X^2=12.629$ ,  $df=1$ ,  $P=0.0011$ ,  $Pcor=0.0022$ ; OR=2.119 95%CI 1.383-3.25). The CC genotype of CAT -262C/T locus were associated with higher risk of chronic airway disease in children (70.74% vs 56.67% in control;  $X^2=9.143$ ,  $P=0.0034$   $Pcor=0.0068$ ; OR=1.85 95%CI 1.23-2.78).

**P06.078****The association between gene polymorphism of cytochrome P450 2D6 and behavioral characteristics**V. A. Shleptsova<sup>1</sup>, J. Shchegolkova<sup>2</sup>, A. G. Tonevitsky<sup>3</sup>;<sup>1</sup>Faculty of basic medicine, Moscow State University, Moscow, Russian Federation,<sup>2</sup>Biological faculty, Moscow State University, Moscow, Russian Federation,<sup>3</sup>Russian research institute of sport and physical education, Moscow, Russian Federation.

The cytochrome P450 2D6 (CYP2D6) enzyme is involved in the hepatic metabolism of many drugs and other exogenous substances. There are a little evidences that CYP2D6 involves in metabolism of endogenous psychoactive substances and expresses not only in liver but also in the brain. Therefore it may influence on psychological process. It has been shown that there are significant differences in personality between extensive and poor metabolizers.

In our investigation we investigated an association of gene polymorphism of CYP2D6 and personality traits. 160 healthy subjects took part in the study (women - 82, men - 78). There were tested by different psychological questionnaires which examine aggression, impulsivity, anxiety and others and also by psychophysiological measurements. We genotyped with PCR-method any gene variations of CYP2D6: CYP2D6\*1, CYP2D6\*2 (C2850T), CYP2D6\*4 (G1934A), CYP2D6\*10 (C100T) We showed that CYP2D6 poor metabolizers (CYP2D6\*4, CYP2D6\*10) had significantly higher level of verbal aggression ( $p=0.04$ ) and inability of stopping aggression ( $p=0.04$ ) and had more pleasure of aggression ( $p=0.02$ ) than extensive metabolizers (CYP2D6\*1, CYP2D6\*2). Furthermore, this relationship expressed to the same extent both at men, and at women.

Association between CYP2D6 gene polymorphism and personality characteristics can indicate on influence cytochrome activity on neuromediator's metabolism in brain.

**P06.079****Cytokine genes polymorphisms are associated with essential hypertension in Tatars from Bashkortostan, Russia**Y. R. Timasheva<sup>1</sup>, T. R. Nasibullin<sup>1</sup>, A. N. Zakirova<sup>2</sup>, O. E. Mustafina<sup>1</sup>;<sup>1</sup>Institute of Biochemistry and Genetics, Ufa, Russian Federation, <sup>2</sup>Bashkir State Medical University, Ufa, Russian Federation.

Cardiovascular disease is the leading cause of death in the Western world. Recently inflammation was proved to be the main substrate underlying the development atherosclerosis. Cytokines mediate interactions of all cells participating in atherogenesis. However, the role of certain cytokines genetic variants on disease risk is not well understood. We tested the hypothesis that specific genetic polymorphisms of some cytokines are associated with increased risk of essential hypertension (EH) and its cardiovascular complications.

355 patients with EH and 343 unrelated normotensive individuals without family history of cardiovascular disease were enrolled in the study. Both patients and control originated from Tatar ethnic group from Bashkortostan, Russia. DNA was isolated from peripheral venous blood. Genotyping was performed using polymerase chain reaction and restriction analysis. Statistical analysis was performed using Fisher's exact test. Odds ratios with 95% confidence interval were also calculated.

We have shown that *IL-10* -627\*C/\*C genotype is associated with decreased hypertension risk (OR=0.53, CI: 0.22-0.78). *TNFA* -308\*G/\*G was found to be protective against stroke in hypertensive patients (OR=0.48, CI: 0.24-0.97). 1159\*A/\*A *IL12B* genotype was also associated with lower stroke risk (OR= 0.43, CI: 0.21\_0.9).

We demonstrate that common genetic variants of *IL10*, *TNFA* and *IL12B* genes are associated with the risk of EH and its complications. Our data suggest a role for cytokine genes polymorphisms in cardiovascular disease.

**P06.080****Strong linkage disequilibrium for the frequent *GJB2* 35delG mutation in the Greek population**H. Kokotas<sup>1</sup>, L. Van Laer<sup>2</sup>, M. Grigoriadou<sup>1</sup>, V. Iliadou<sup>3</sup>, J. Economides<sup>4</sup>, S. Pomoni<sup>1</sup>, A. Pampanos<sup>1</sup>, N. Eleftheriades<sup>5</sup>, E. Ferekidou<sup>6</sup>, S. Korres<sup>6</sup>, A. Giannoulia-Karantana<sup>7</sup>, G. Van Camp<sup>2</sup>, M. B. Petersen<sup>1</sup>;<sup>1</sup>Institute of Child Health, Athens, Greece, <sup>2</sup>University of Antwerp, Antwerp, Belgium, <sup>3</sup>AHEPA Hospital, Thessaloniki, Greece, <sup>4</sup>Aghia Sophia Children's Hospital, Athens, Greece, <sup>5</sup>St. Loukas Hospital, Thessaloniki, Greece, <sup>6</sup>Athens

University, Athens, Greece, <sup>7</sup>Athens University Medical School, Athens, Greece. Approximately one in 1,000 children is affected by severe or profound hearing loss at birth or during early childhood (prelingual deafness). Up to forty percent of autosomal recessive, congenital, severe to profound hearing impairment cases result from mutations in a single gene, *GJB2*. The 35delG mutation accounts for the majority of *GJB2* mutations detected in Caucasian populations and represents one of the most frequent disease mutations identified so far. Some previous studies have assumed that the high frequency of the 35delG mutation reflects the presence of a mutational hot spot, whilst other studies support the theory of a common founder. Greece is amongst the countries presenting high frequency of the 35delG mutation (3.5%), and a recent study raised the hypothesis of the origin of this mutation in ancient Greece. We genotyped 60 Greek deafness patients homozygous for the 35delG mutation for six single nucleotide polymorphisms (SNPs) and two microsatellite markers, mapping within or flanking the *GJB2* gene, as compared to 60 Greek hearing controls. A strong linkage disequilibrium was found between the 35delG mutation and markers inside or flanking the *GJB2* gene, at distances of 34 kb on the centromeric and 90 kb on the telomeric side of the gene, respectively. Our study supports the hypothesis of a founder effect and we further propose that ethnic groups of Greek ancestry could have propagated the 35delG mutation, as evidenced by historical data beginning from the 15<sup>th</sup> century BC.

**P06.081****Detoxification system gene variants and small-for-gestational-age births**N. Nabieva<sup>1</sup>, T. Ivashchenko<sup>2</sup>, N. Shabalov<sup>1</sup>, V. Baranov<sup>2</sup>;<sup>1</sup>Medical Pediatrics Academy, St.Petersburg, Russian Federation, <sup>2</sup>Ott's institute of Obstetrics and Gynecology, St.Petersburg, Russian Federation.

Small-for-gestational-age (SGA) is defined as birth weight below the 10th percentile according to gestational age and sex based on national standards. Little is known about the role of detoxification system gene variants as risk factors for SGA births. Only a few studies have considered the direct role of polymorphic xenobiotic-metabolizing genes in fetal growth. GSTM1 and GSTT1, in the GST family, are both involved in the biotransformation of a wide range of reactive toxic and mutagenic compounds, including ROS oxygen species and components of tobacco smoke. GST enzymes are present in large amounts in the placenta in early pregnancy and are expressed early in embryonic development. The polymorphisms of xenobiotic-metabolizing genes (GSTM1, GSTT1, NAT2) responsible for xenobiotics conjugating enzymes of Phase II detoxification system were studied by PCR-RFLP in SGA infants and control group newborns.

The genotypes distribution for NAT2 gene was identical in controls group and in group of SGA patients. The analysis of genotypes distribution for polymorphism and GST M1 in patients and in controls has not revealed significant differences.

The frequency of GSTT10/0 genotype was significantly higher in group of patients compared to controls (34% versus 10% respectively,  $p=0.003$ ). Concordance of both GSTT1 0/0 and NAT2 S/S genotypes were found in 17.2% of patients and was almost 4 times more compared to only 4% in control ( $p=0.01$ ). The 37% of patients had at least two functionally impaired genotypes for studied genes.

The study provides new information on the role of polymorphic detoxification genes in development of SGA.

**P06.082****Analysis of the association between LECAM-1 P213S polymorphism and ESRD in diabetic patients**

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Mutations in lyam-1 gene (1 q23-q25) may predispose to phenotypes that can aggravate the evolution of vascular complications in diabetic patients, including renal disease.

The purpose of this case-control study was to estimate the association of LECAM P213S polymorphism with end-stage renal disease (ESRD) in diabetic patients.

Clinical information and biological samples were collected from diazylized type I diabetic patients ( $n=100$ , M:F = 50:50) and healthy subjects ( $n=200$ , fasting glycemia  $93.2\pm8.2$  mg/dl). Healthy subjects were

selected to be matched for age, gender and with subjects with T1DM patients. All subjects selected for this study were unrelated Romanian Caucasians. DNA samples were used for genotyping LECAM-1 P213S polymorphism using restriction of amplicons with Hph1 endonuclease.

The distribution of LECAM P213S in all lots is in agreement with Hardy - Weinberg equilibrium. When compare the distribution of LECAM genotypes in patients and control lots we observed only a trend of association with ESRD in T1DM patients. The sex or age at ESRD onset seems to not modify these observations. The lack of association with ESRD in T2DM seems to be not similar with results reported for other populations.

In conclusion, our study showed only a trend of association between LECAM P213S polymorphism and IRC in DM 1 patients.

#### P06.083

#### On the choice of an exposure to test for gene-environment interactions in type 2 diabetes: A new genotype-free method!

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Gene-environment interactions might be involved in the susceptibility to multifactorial diseases but are difficult to detect. Available methods to test for gene-environment interactions usually concentrate on some particular genetic and environmental factors. Rather than focusing on a known genetic factor, we applied a new method to determine whether or not a given exposure is susceptible to interact with unspecified genetic factors, using the degree of familial aggregation as a surrogate. The Odds Recurrence Ratio (ORR) is an indirect measure of interaction since it contrasts recurrence risks in sibs of affected indexes when stratifying on the exposure of indexes. A Wald chi-square test based on the estimate of the ORR and its variance tests for the gene-environment interaction, while accounting for a possible confounding bias if indexes and their sibs are correlated for the exposure. An application on a sample of 588 nuclear families ascertained through one index affected with type 2 diabetes is presented where gene-environment interactions involving obesity, physical activity and dietary fat intake are investigated. An association with obesity is clearly evidenced and a potential interaction involving this factor is suggested ( $p=0.06$ ). Multiple sibships have been used to increase sample size but a permutation procedure is needed to account for dependency of sibpairs. Results of this undergoing work will also be presented. The method proposed here might be of particular interest prior to genetic studies to help determine the environmental risk factors that will need to be accounted for and select the most appropriate samples to genotype.

#### P06.084

#### Compound heterozygosity in DJ-1 gene non-coding portion related to parkinsonism

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Mutations in DJ-1 gene cause a clinically characteristic autosomal recessive juvenile onset form of Parkinson's disease (PD). We sequenced the DJ-1 gene in 40 sporadic patients with early onset Parkinson's disease and 100 appropriate controls, originated from southern Italy. We identified a single patient with age at onset of 38 years carrying two novel heterozygous mutations, both located in non coding regions. The first mutation (g. 159 C/G), located in the promoter region, was inherited from his mother whereas the second mutation, an insertion in the intron 4 splice site (IVS4+3 insA), was transmitted from his father. The DJ-1 cDNA level both in the patient and in a control subject was normalized with the GAPDH gene and a significant reduction ( $P=0.027$ ) was found in the patient. Moreover, we obtained a single size of DJ-1 cDNA fragments in both wild type and mutated individuals. Of interest, no family member carrying only one of the two new mutations manifested symptoms of EOPD. Genomic rearrangements were excluded. Both mutations were absent in 200 control chromosomes.

In the remaining PD patients, we did not detect any pathogenic DJ-1 mutation. Our findings indicate that the mutant alleles are expressed at a lowered level, or that their corresponding mRNAs are partially degraded. Although the finding of a single size cDNA fragment is not suggestive of the activation of any alternative cryptic splice site, this cannot be fully excluded. Further studies are in progress.

#### P06.085

#### A genome-wide association study in schizophrenia using DNA pooling on 574 parent-offspring trios

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We conducted a genomewide association study (GWAS) on schizophrenia with DNA pooling in order to reduce the cost of the project. We used a parent-offspring trios design in order to avoid the potential problems of population stratification. We constructed pools from 605 unaffected controls, 574 SZ patients and a third pool from all the parents of the patients. We hybridised each pool 8 times on Illumina Human-Hap550 arrays. We estimated the allele frequencies of each pool from the averaged intensities of the arrays. The significance level of results in the trios sample was estimated on the basis of the allele frequencies in cases and non-transmitted pseudocontrols, taking into account the technical variability of the data. We selected for individual genotyping the highest-ranked SNPs, after excluding poorly performing SNPs and those that showed a trend in the opposite direction in the control pool. We genotyped 63 SNPs in 574 trios and analysed the results with the transmission disequilibrium test (TDT). 40 of those were significant at  $p<0.05$ , with the best result at  $p=1.2 \times 10^{-6}$  for rs11064768. This SNP is within the gene CCDC60, a coiled-coil domain gene. The most interesting result was for the third-best SNP: rs893703 ( $p = 0.00016$ ), within RBP1, a candidate gene for schizophrenia.

#### P06.086

#### DNase1 Exon2 analysis in Tunisian patients with Rheumatoid Arthritis, Systemic Lupus Erythematosus and Sjögren Syndrome and healthy subjects

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Autoimmune Diseases (AID) are caused by the loss of immunological tolerance against self-antigens. The DNASE1 seems to participate in the genetic susceptibility of some AID, particularly the Systemic lupus erythematosus (SLE). Deficiencies in the normal rate of removal of chromatin or chromatin-protein complexes contribute to the development and severity of SLE. In fact, two mutations were reported among SLE patients from Japan and Spain (the 172 A→T mutation (K5X) and the 46-72 deletion respectively). In the aim to evaluate the DNASE1 contribution in the genetic susceptibility of Rheumatoid arthritis (RA), Sjögren syndrome (SS) and SLE in Tunisia, we studied these two mutations by PCR RFLP and by fragment size analyses on 3% agarose gel electrophoresis respectively. In order to achieve this work, DNA from 151 patients affected with RA, 55 patients affected with SS, 34 patients affected with SLE and 232 healthy control subjects were explored. Both mutations were absent among patient and control subjects. In addition, by direct sequencing, the DNASE1 exon 2 was analysed among 26 control subjects to identify a possible new polymorphic variations. Five known SNPs were explored (rs8176921, rs8176922, rs8176927, rs8176930, rs34907394). The 2113 G→T SNP (rs8176927:Arg2Ser) is the only polymorphic functional nonsynonymous SNP. By PCR-RFLP, patient and healthy subjects DNAs were genotyped for rs8176927 for a case-control design. The statistical analysis showed no significant differences between patients and controls genotype data.

In conclusion, by analysing DNASE1 Exon2 gene, our study showed no particular genetic involvement of this gene in SLE, RA and SS development.

**P06.087****Genetic effect of DAT1 and DRD2 gene polymorphisms on personality traits in healthy individuals from Russia**

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Psychobiological model proposed by Cloninger supposes that sociability related personality traits are mediated by dopaminergic system functioning. We aimed to define a single genotype effect of *DRD2 TaqIA* and *DAT1 Mspl* polymorphisms and to check possible epistatic effect between them and personality traits (assessed with the EPI and TCI questionnaires). 602 healthy individuals (men-206, women-396) of Caucasian origin (Russians-214, Tatars-388) were recruited from Russia (mean age $\pm$ SD, 19.85 $\pm$ 2.43 years).

MANOVA (carried out with gender and ethnicity as second factors) revealed the influence of *DRD2*\*gender interaction on Novelty Seeking (NS) ( $p=0.017; F=5.734$ ) and Reward Dependence (RD) ( $p=0.039; F=4.298$ ). Multiple comparisons explained this interaction by the differences in NS and RD scores between female carriers of A1-allele and male carriers of A2/A2-genotype ( $p=0.001; F=11.208$  and  $p=0.000; F=16.464$  correspondingly); in NS scores between females with A2/A2-genotype and males with A1-allele ( $p=0.000; F=18.678$ ). Demonstrated effect could be partly due to higher NS and RD in women compared to men ( $p=0.000; F=26.643$  and  $p=0.001; F=21.102$ ). Moreover, association of A1-allele with lower NS ( $p=0.040; F=4.278$ ) and higher RD ( $p=0.029; F=4.814$ ) was demonstrated in men. The effect of *DAT1*\*ethnicity interaction was observed on RD ( $p=0.018; F=5.615$ ) caused by the differences in RD of G/G-genotype carriers from Tatar and Russian population ( $p=0.001; F=10.752$ ). Association between *DAT1* A-allele carriers and higher extraversion ( $p=0.045; F=4.030$ ) and NS ( $p=0.042; F=4.148$ ), lower Persistence ( $p=0.020; F=5.401$ ) was demonstrated.

Our findings suggest single *DAT1* gene effect on extraversion and Persistence, while differences in NS and RD are caused by *DRD2*\*gender and *DAT1*\*ethnicity interaction.

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**P06.088****The T-Allele of the Dopamine Transporter Core Promoter Polymorphism and Risk of Attention-Deficit Hyperactivity Disorder**

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Pharmacological and genetic findings implicate the DAT1 gene in the development of attention-deficit/hyperactivity disorder. In this study, we examined whether either allele of the DAT1 core promoter -67 functional polymorphism is associated with ADHD in a case/control study. The allele and genotype frequencies of the polymorphism were studied in 110 patients and 120 controls, which were matched on the basis of sex, age and ethnicity. The genotype frequencies in the patients group were as follows: AA 19.2%; AT 65.2%; TT 15.4% vs. the genotype frequencies in the control group: AA 47.5%; AT 43.3%; TT 9.2% [ $\chi^2=20.73$ , df = 2] The T-allele of the -67A/T polymorphism revealed a ~1.56-fold excess in the patients group comparing with the controls [ $\chi^2=14.50$ , df = 1]. For the first time, these findings provide tentative evidence of the contribution of the DAT1 gene core promoter polymorphism to the etiopathophysiology of ADHD at least in the Iranian population that we have studied. Further work is warranted to confirm this finding and to assess its generalization to other ethnic groups.

**P06.089****Origin of nondisjunction in trisomy 21 Down syndrome**

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Down syndrome due to trisomy 21 is the most common human chromosomal abnormality. In order to gain further insight into the mechanism underlying nondisjunction, we investigated the association between reduced recombination and nondisjunction. We genotyped 12 microsatellite markers spanning along 21q from centromere to telo-

mere in 122 individuals with free trisomy 21 and in their parents. Our DNA marker studies of parental origin were informative in 119 families with overwhelming majority 89.91% being maternal and 10.09% is paternal. Only cases of maternal origin were included in our analysis. The distribution of nondisjunction in maternal meiotic I and meiotic II stages were 81.19% and 19.81% respectively. The mean maternal age of nondisjunction in our Indian population sample is 27.58 $\pm$ 6.4 which is significantly lower than that of Caucasians. We created a genetic map, using maternal meiotic I nondisjunction data. The female genetic map was restricted to 21q. The distribution of chiasma shows a difference throughout the length of chromosome arm(21q) with more recombination towards telomeric end in comparison to control data. The telomeric exchange is a significant risk factor for meiotic I nondisjunction, irrespective of the age of the mother. Analysis of crossover events indicates that in younger mother (< 29) there was an increase in both zero- and one exchange events, suggesting reduction of recombination. The linkage map of 21q(39.58cM) was significantly shorter than the control female linkage map, indicating an overall reduction of recombination. Thus, reduced recombination may be responsible, at least in part, for the etiology of nondisjunction in trisomy 21.

**P06.090****Identification of a new locus for autosomal recessive Dyschromatosis universalis hereditaria on chromosome 12q21-q23**

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Background: Dyschromatoses are a group of pigmentary dermatoses characterized by the presence of small and irregularly shaped hyper- and hypopigmented maculae. There are two major forms of the disease: Dyschromatosis symmetrica hereditaria (DSH), where the maculae are restricted to the dorsal aspects of the extremities, and Dyschromatosis universalis hereditaria (DUH), where patients are affected by a generalized distribution of the maculae over most of their body. Usually, both disorders show autosomal dominant inheritance, but in some cases autosomal recessive inheritance was reported. Autosomal dominant DSH was mapped to chromosome 1q21.3, and mutations in the *ADAR*- (DSRAD-) gene were identified. A second dyschromatosis locus was mapped on chromosome 6q24.2-q25.2, but the two analyzed families, which were initially reported to be affected with DSH, were later suggested to have autosomal dominant DUH.

Patients and methods: We investigated whether one of the two known Dyschromatosis-loci is involved in the development of DUH in a consanguineous family from Saudi Arabia (four siblings were affected, three siblings and the parents were unaffected).

Results: After confirmation that neither of the two known loci is linked to the disease in this family, a SNP-based genome-wide linkage analysis was performed and a new locus for dyschromatosis was identified on chromosome 12q21-q23. The candidate region (LOD score of 3.4) contains 125 known or predicted genes.

Conclusion: We have identified a new locus for DUH, and obtained evidence that DUH and DSH are distinct disorders with different genetic origins.

**P06.091****Frequency of the coding polymorphisms in the PARK2 gene - characterization of the polish group with Parkinson disease of the early onset**

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Parkinson disease (PD; OMIM 168600) is the second most frequent neurodegenerative disease in the elderly. Among the PD patients there is less common group of age of onset before 50 years classified as a early-onset PD (EO-PD) with mutations in parkin gene (PARK2; OMIM 62544) as a common cause. The prevalence of this form of PD is not known. However, in Europe, parkin type of EO-PD accounts for approximately 50% of autosomal recessive parkinsonism and 18% of

individuals without a family history with onset before age 45 years. A variety of deletional and point mutations has been described in PARK2 gene as well as a few coding polymorphism being a possible risk factors for sporadic and familial PD. The role of this polymorphisms is still unclear and published results contradictory.

We present the analysis of the frequency of the four coding polymorphism detected in the group of EO-PD sporadic patients of Polish origin (Ex4 S167N, Ex10 V380L, Ex 11 D394N and R402C) in comparison with the group of control subject.

Presented results are preliminary as the groups are not big enough to be conclusive (70 EO-PD patients, 100 control subjects) but they indicate no difference in frequency of analysed polymorphisms in both of them. We can conclude there is no association of any of polymorphism under consideration with sporadic EO-PD form.

#### P06.092

#### Genetic association of single SNPs and a LD-Haplotype at PSORS6 in patients with early onset psoriasis and evidence for epistasis with PSORS1 risk locus

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Psoriasis is a genetically complex, chronic inflammatory skin disease. We have previously performed a genome wide linkage study in a set of psoriasis families and have identified a susceptibility locus on chromosome 19p13 (PSORS6). In a follow-up linkage disequilibrium (LD) study in an independent family based cohort, we found evidence for association to two newly discovered microsatellites at this locus (D19SPS20: P < 2.7\*10<sup>-2</sup>, D19SPS21: P < 5.3\*10<sup>-5</sup>). An association scan in 300 trios, based on the LD structure of the region, revealed association to several single SNPs in one LD block. When we stratified this cohort for carrying the PSORS1 risk allele at the HLA-C locus on chromosome 6p, evidence for association became much stronger at single SNP and haplotype levels (p-values between 2.0\*10<sup>-4</sup> and 9.0\*10<sup>-4</sup>). In a population based replication study of 1,114 psoriasis patients and 937 control individuals, evidence for association was observed again after stratification to the PSORS1 risk allele. In both study groups, logistic regression showed evidence for interaction between the risk alleles at PSORS1 and PSORS6. The associated LD block did not comprise any known genes. Interestingly, an adjacent gene, MUC16, coding for a large glycosylated protein expressed in epithelia, could be shown to be also expressed in tissues relevant for pathogenesis of psoriasis such as skin and thymus. In summary, we confirmed and refined the susceptibility locus at PSORS6 which seems to be restricted to patients with early onset psoriasis carrying the PSORS1 risk allele.

#### P06.093

#### Parkin, DJ-1 and PINK1 mutations in Dutch patients with early-onset Parkinson's disease

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**Objectives** Recessively inherited early-onset Parkinson's disease (EOPD) has been associated with mutations in the *Parkin*, *DJ-1* and *PINK1* genes. In order to assess the genetic contribution of all known recessive genes in EOPD in the Dutch population we investigated the prevalence and nature of mutations in the PROPARK (PROfiling PARKinson's disease) patient cohort. **Methods** A total of 186 unrelated Dutch EOPD patients (mean age at onset: 41.1 ± 6.6 years, 130 sporadic, 56 familial) with an age at onset (AAO) ≤ 50 years were studied. The genetic screening was performed by direct sequencing and dosage analysis of the three genes. **Results** Mutations were found in 9% (16/186) of the patients however PD may be explained in only six (3%) patients who carried homozygous or heterozygous compound mutations in *Parkin* (n=5) or *DJ-1* (n=1). No homozygous or compound heterozygous mutations were detected in *PINK1* gene. In addition, 6 (3%), 3 (2%) and 2 (1%) patients carried a single heterozygous mutation in *Parkin*, *DJ-1* and *PINK1* genes, respectively. Interestingly, two

novel mutations were found in one patient in heterozygous state but not in 700 ethnically matched control chromosomes. **Conclusions** *Parkin* was the most frequently mutated gene in EOPD, followed by *DJ-1* and *PINK1*. The low overall mutation frequency observed indicates that caution has to be taken with the extrapolation of mutation frequencies found in other studies and populations and suggests that other genes and risk factors for PD remain to be discovered.

#### P06.094

#### DNER, a neuronal transmembrane protein is dispensable for gross cellular morphology and sensory nerve fiber excitability

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DNER is the first transmembrane protein expressed in the brain containing only multiple epidermal growth factor (EGF)-like repeats in its extracellular domain. DNER further contains a signal peptide, a serine rich stretch, a transmembrane domain and a cytoplasmatic C-terminus. Since its close relation to Notch and Delta it has been named Delta/Notch-like-related receptor. Structural homology to other EGF-bearing proteins involved in extracellular signalling events and in neuronal development, point out a possible involvement of DNER in such processes.

The extracellular domain of DNER was cloned and recombinant protein was used for polyclonal antibody production. Recombinant and full length protein is larger than the theoretical calculated mass and glycosidase digestion indicate that DNER is glycosylated. Distribution analysis by semi quantitative RT-PCR show that almost no mRNA was present in non-neuronal tissues. In mouse embryos, DNER mRNA is detectable at day 9.5 of gestation in the neural tube, dorsal root ganglia, ear placode, anterior and posterior zones of the developing forelimb, and in different cell types of the retina. The distribution of DNER protein is also mainly restricted to neuronal tissues and can also be detected in peripheral ganglia and nerves. A knock-out targeting vector was generated and the homozygote animals are being analyzed. In the homozygote knock out animals no DNER protein was detected. Surprisingly, the mice are viable and show no obvious phenotype. The gross cellular morphology appears unchanged and further analysis of the peripheral nerve functions, reveal no effects in excitability of specific A-fibers.

#### P06.095

#### Polymorphisms in ESR1 and ESR2 genes are associated with susceptibility to endometriosis

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**Introduction.** Endometriosis is defined as the presence of endometrial-like tissue outside the uterus. It affects about 5-10% of women of reproductive age and causes dysmenorrhoea, abdominal pain, dyspareunia and infertility. Endometriosis is considered to be an estrogen-dependent disease with a genetic background. The aim of this study was to evaluate possible associations between endometriosis and polymorphisms in the estrogen receptor α (*ESR1*) and β (*ESR2*) genes.

**Materials and methods.** 123 women (age: 18-44 years) with surgically confirmed endometriosis were enrolled in the study. 200 fertile women (at least two children; age: 30-50 years) from general population were used as controls. The 397 T/C polymorphism in the first intron of the *ESR1* gene was determined by PCR-RFLP analysis, using the restriction endonuclease *Pvu*II. The number of CA repeats in the fifth intron of the *ESR2* gene was detected with fragment analysis.

**Results.** The distribution of *ESR1* TT/TC/CC genotypes was 17.9/54.5/27.6% among patients and 30.0/51.0/19.0% in the control group ( $p=0.028$ ;  $\chi^2$ -test). The T/C allele frequencies were 45.1/54.9% and 55.5/44.5%, respectively ( $p=0.008$ ). The number of CA repeats in the *ESR2* gene ranged from 14 to 26. The *ESR2* alleles were classified as short and long, with ≤21 and >21 repeats, respectively. Endome-

triosis patients had short (CA)n alleles more frequently than healthy controls (61.4% vs 51.5%,  $p=0.018$ ). This difference was even more remarkable in case of stage IV disease (80.8% vs 51.5%,  $p=0.007$ ). Conclusions. The C-allele of Pvull polymorphism in *ESR1* gene and short *ESR2*-(CA)n alleles could be associated with susceptibility to endometriosis.

#### P06.096

#### Genetic polymorphisms and physical performance in endurance sports

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Genes determine the potential for developing many of the structural and functional characteristics important in determining sports performance. So, the genetic constitution defines innate qualities. The genes also determine the speed and extent to which the performance characteristics of the individual respond to training, diet, and other environmental factors. The effects of genes on response to training to endurance sports seem to be moderate to large.

Here we study the correlation between endurance sports and the following polymorphisms: insertion/deletion in intron 16 (I/D) within ACE gene; p.C282Y and p.H63D in HFE (hemocromatosis) gene and p.R577X within ACTN3 (alpha3-actinin).

We have genotyped samples of two groups of endurance sportsmen (runners n=48 and cyclists n=60) and control population (n=66).

We have observed that the D allele of the ACE gene were more frequent in sportsmen as a whole (72%) than in control population (42.4%)  $p=0.0001$ . For runners the frequency were 70.7%  $p=0.003$ , and for cyclist 72.9% with  $p=0.004$ .

p.63D allele of HFE was present in 60.3% of the cyclist and in 41.3% of the controls ( $p=0.006$ ). There were no differences between runners and controls. The frequency of the p.282Y allele is very low in our population, nevertheless, it was more frequent in cyclist than in runners  $p=0.015$

The study of ACTN3 gene did not show differences between the groups.

We conclude that genetic constitution contributes to the talent to practice endurance sports, and that the "predisposing" genes seem to be sport-specific.

#### P06.097

#### Analysis of ACE and eNOS gene polymorphisms in hemodialysis patients

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Polymorphisms in genes encoding for angiotensin I converting enzyme (ACE) and NO synthase (eNOS) have been reported to be associated with increased susceptibility to renal failure. The aim of the study was to investigate allelic distribution of eNOS (4a4b, G894T) and ACE (I/D) gene polymorphisms in hemodialysis patients (n=128) and control group (n=101) and to find possible association of mutant allele with this disorder in Serbian population. The polymorphic variants were analysed using PCR-RFLPS and PCR-VNTR method.

The allele frequencies of two eNOS gene polymorphisms were: 0.27 894T, 0.21 4a, in the group of patients and 0.31 894T, 0.17 4a in control group. The frequencies of G894T polymorphism genotypes were 0.56 GG, 0.35 GT, 0.09 TT in the group of patients and 0.50 GG, 0.38 GT, 0.11 TT in the control group. The genotype frequencies of eNOS inton 4 polymorphism were 0.65 bb, 0.28 ba, 0.07 aa in the group of patients, and 0.70 bb, 0.26 ba, 0.04 aa in the control group. The difference between allelic and genotype frequencies (patients/control) is not statistically significant. The allele frequencies of ACE gene were 0.71 D (genotype: 0.58 DD, 0.28 ID, 0.14 II) in the group of patients and 0.62 D (genotype: 0.45 DD, 0.34 ID, 0.21 II) in the control group. The D allele frequencies in a group of patients is significantly higher than in control group ( $\chi^2=3.96$ , DF=1,  $p<0.05$ ).

Thus, ACE D allele can be considered as genetic marker associated with renal failure, independently of alleles 4a or 894T.

#### P06.098

#### Genetic analysis of autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE)

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Epilepsy is a heterogeneous group of disorders affecting 0.5-1% of the population worldwide. Among them, autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an idiopathic epilepsy characterized by nocturnal motor seizures that typically begin during childhood. Familial cases follow an autosomal dominant pattern of inheritance with reduced penetrance. Disease-causing mutations have been described in the genes encoding the neuronal nicotinic acetylcholine receptor subunits *CHRNA2*, *CHRNA4*, and *CHRN2*. A fourth locus has been identified on chromosome 15q24, and putative susceptibility loci mapped to chromosomes 3 and 8. These genes/loci account for only a minority of ADNFLE cases, indicating genetic heterogeneity.

We have ascertained two new pedigrees segregating ADNFLE. Family 1 is a five-generation pedigree originating from Chile, with twelve affected subjects and three obligate carriers. Patients present with focal, motor seizures or secondary generalized attacks. Family 2 is a three-generation Spanish family with four affected relatives, including one patient with concomitant early-onset Parkinson disease and a second patient with West syndrome. In order to identify the underlying disease-gene(s), we are currently performing cosegregation analysis using markers spanning all known ADNFLE loci. In addition, we are also studying other acetylcholine receptor genes (*CHRNA3*, *CHRNA5*, *CHRNA7* and *CHRN4*), and loci on chromosomes 10 and 22, responsible for partial epilepsy with auditory features and familial partial epilepsy with variable foci, respectively. In the event that all loci are excluded as the underlying cause of disease, we will perform a genome-wide scan to identify a novel gene responsible for the ADNFLE phenotype in family 1.

#### P06.099

#### Association between genetic polymorphisms and pediatric essential hypertension

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Recent whole genome association studies identified a number of loci associated with the development of different complex trait life style diseases, i.e. coronary heart disease, diabetes type 2 and many others. Because these diseases increasingly affect children it can be assumed that similar genetic factors may contribute to their development in pediatric cases. This study presents association analysis for three common SNPs reported to be associated with coronary heart disease/obesity/diabetes type 2 in adults in a sample of children diagnosed with essential hypertension.

A total of 166 families were included in the study (514 individuals)... Allele specific PCR was adopted for the study. Polymorphism rs9939609 from the gene FTO, polymorphism rs7566605 from the gene INSIG2 and polymorphism rs10757278 were selected for the analysis.

Weak association between polymorphisms rs10757278, rs9939609 and pediatric essential hypertension was observed. Also strong association for polymorphisms rs7566605 was present in our sample of families with children affected with pediatric hypertension.

Our results suggest that the three polymorphisms might be associated with the development of essential hypertension in children. The rs7566605 from the gene INSIG2 may be involved in the pathogenesis of pediatric essential hypertension.

**P06.100****An association between essential tremor and ETM2 locus in Latvian population**

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Essential tremor (ET) is one of the most common neurological disorders in humans. An autosomal dominantly inherited form of ET is genetically linked to two loci on chromosomes 3q13 (ETM1) and 2p24.1 (ETM2) in families from different parts of the world. Numerous of candidate genes for ET disorder - HS1-BP3, HCLS1, DRD3 - have been suggested during last years.

Here we report study analysing a group of 104 unrelated Latvian patients with ET for a genetic association with loci in candidate regions ETM1 and ETM2. Out of them, 52 were classified as familial on the bases of familial history. All patients were genotyped using sixteen informative STR markers within two loci associated with ET (9 markers for ETM2 and 7 for ETM1) and statistically analysed. Allele frequencies were estimated on 97 normal controls, matched by age and gender. The one concrete allele frequencies were significantly different between familial ET samples in case of marker D2S220 ( $p=0.0281$ ) and total ET samples in case of marker D2S2201 ( $p=0.0385$ ) in comparison to control samples. Other loci did not show significant allele frequency differences between familial cases, total ET cases or control groups. In addition, 7<sup>th</sup> exon of HS1-BP3 gene containing A265G substitution and 1<sup>st</sup> exon of DRD3 gene containing S9G substitution were analysed in all ET patients and control samples. Analysed data did not reveal any association between known variants of HS1-BP3 and DRD3 genes and ET phenotype.

Our data suggest an association between ETM2 locus and familial essential tremor in Latvian population.

**P06.101****Lysyl Oxidase-like 1 gene Polymorphisms in Exfoliation Syndrome, Exfoliation Glaucoma and Primary Open Angle Glaucoma in the Finnish Population**

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Exfoliation syndrome (XFS) is an age-related ocular disorder and a risk factor for the development of glaucoma. XFS is characterized by abnormal accumulation of greyish fibril-like material in the anterior segment of the eye. Similar material has also been found in extraocular tissues. XFS is prevalent worldwide but the prevalence varies widely in different populations; being even 20%-40% among individuals >80 years in Scandinavian countries. Familiar aggregation studies suggest genetic contribution to XFS. In a recent genome-wide association study, three SNPs, (rs2165241, rs1048661 (R141L) and rs3825942 (G153D)) on lysyl oxidase-like 1 (LOXL1) gene were found to be strongly associated with XFS and XFG, in Icelandic and Swedish patients. Together two non-synonymous SNPs accounted for 99% of XFG in this population. These two SNPs were in complete LD and three haplotypes were observed. Haplotypes G/G (OR=27.05) and T/G (OR=8.90) were risk haplotypes relatively to G/A with lowest estimated risk. In this study we investigate whether three LOXL1 SNPs are associated with XFS/XFG in Finnish population. SNPs are screened by direct-sequencing in XFS-, XFG- and POAG- patients (100/group) and as controls in ~120 individuals without any sign of XFS/XFG/POAG and ~300 Finnish blood donors. In preliminary results 59% (30/51) of XFS patients, 69% (40/58) of XFG patients and only 18% (10/55) of POAG patients were homozygous for the highest risk haplotype GG. Likewise in Icelandic and Swedish population about 25% (82/325) of general population in Finland were homozygous for the haplotype GG. Studies are ongoing and more XFS/XFG/POAG patients will be analyzed.

**P06.102****FADS genotypes and desaturase activity estimated by arachidonic to linoleic acid ratio are associated with inflammation and coronary artery disease**

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**Background:** The delta-5 and delta-6 desaturases, encoded by FADS1 and FADS2 genes, are key enzymes in the conversion of linoleic acid (LA) to arachidonic acid (AA). AA is the precursor of a cascade of mediators, such as eicosanoids, with inflammatory properties. Single nucleotide polymorphisms (SNPs) in FADS1 and FADS2 have been associated with different levels of AA and LA, with possible functional consequences on desaturase activity.

**Methods and Results:** Thirteen FADS SNPs and AA/LA ratio on red blood cell (RBC) membranes, a marker of desaturase activity, were evaluated in 876 subjects with (n=610) or without (n=266) angiographically documented coronary artery disease (CAD). AA/LA ratio was higher in CAD patients ( $2.17 \pm 0.41$  versus  $1.99 \pm 0.36$ ;  $P=2.5 \times 10^{-10}$ ), and an increased AA/LA ratio resulted an independent risk factors for CAD (OR 2.22, 95%CI 1.37-3.61 for higher versus lower ratio tertile). Furthermore, hs-CRP levels increased progressively across AA/LA ratio tertiles. In a linear regression model including all the 13 SNPs analysed, 4 resulted to be independent predictors of AA/LA ratio variability. The subjects carrying the highest number of alleles associated with a raised ratio, as well as haplotypes with the highest number of such alleles, presented proportionally more elevated hs-CRP concentrations and an increased probability of having CAD.

**Conclusions:** An increased desaturase activity is associated with an elevated hs-CRP and, in turn, with an increased risk for CAD. Subjects carrying FADS genotypes associated with an higher desaturase activity may be prone to a proinflammatory response favouring atherosclerotic vascular damage.

**P06.103****MLPA PCR-analysis for registration of the most frequent mutations in MEFV gene indigenous to Armenians**

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Familial Mediterranean fever (FMF) is an autosomal recessive disease particularly common in several populations of Mediterranean extraction, and affecting mainly Turks, Jews, Armenians, and Arabs. It is characterized by recurrent short episodes of fever, sterile peritonitis, arthritis, and pleurisy. The gene responsible for FMF, MEFV(MIM# 608107), was identified by positional cloning in 1997. The product of the MEFV gene, named pyrin/marenostrin, is expressed in polymorphonuclear cells and monocytes and it is proposed that it regulates inflammatory responses at the level of leucocyte cytoskeletal organisation. Thirty six mutations located in the MEFV gene have been identified so far, mostly in exon 10, followed by exons 1, 2, 3, 5 and 9.

We elaborated the multiplex system for MLPA PCR-analysis for most frequent mutations indigenous to Armenians M694V, M680I, V726A, F479L, R761H, and E148Q in MEFV gene in exons 10, 5 and 2. The MEFV probe mix contains 18 different probes with amplification products between 82 and 145 bp. Length difference between consecutive amplification products is 3 or 4 bp.

DNA sample from 57 unrelated Armenian FMF patients were examined by this multiplex system. 83 chromosomes with different mutations were revealed So, calculated nformative of this system more then 73% for Armenians living on the Russian Federation territory.

Advantages of this method are: specificity; possibility to work with small quantity of researching material- method speed; for all mutation types used two universal ferment: ligase and polymerase; detection with help of PAAG or capillary electrophoresis; quantitative analysis genes copy or stripes of genes.

**P06.104****Association study of candidate genes in the chromosome 5p13.1-q11.2 linkage region for familial primary cutaneous amyloidosis**

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Primary cutaneous amyloidosis (PCA) is a relatively common skin disorder in South America and Southeast Asia. The pathogenesis of PCA remains unclear. Most cases of PCA are sporadic but familial aggregation has been reported from South America and Taiwan. The different susceptibility among ethnic groups suggests that genetic factor may play an important role in its pathogenesis.

In our previous study, we performed genome-wide linkage analysis of familial primary cutaneous amyloidosis using both microsatellite and Affymetrix GeneChip® Human Mapping 10K Array and identified significant linkage evidence (maximum LOD=4.50) for a portion of FPCA families on chromosome 5. In the candidate region identified by SNP linkage mapping, there are 12 known genes.

To characterize the susceptibility genes for FPCA, we applied the Illumina GoldenGate® Assay to genotype large amounts of functional SNPs and tagSNPs selected from the HapMap project within or around candidate genes of significant linkage region on chromosome 5. A total number of 23 FPCA families with 75 affected and 72 phenotypically normal subjects and 94 normal control subjects were genotyped in the study. Several SNPs on NNT, FGF10, HCN1, ITGA1, ITGA2, NDUFS4, SNAG1 genes demonstrated statistically significant results (p-value <0.05).

In conclusion, our study found positive association between several candidate genes on chromosome 5 and FPCA. Independent study and functional study are required to confirm our findings.

**P06.105****High prevalence of familial pulmonary fibrosis in Newfoundland suggestive of a novel genetic etiology**

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Idiopathic Pulmonary Fibrosis (IPF) is a form of interstitial lung disease that is progressive and often fatal. This devastating disease is limited to the lungs, and the only treatment that improves survival is a lung transplant. It is usually diagnosed between ages 50-70 years. Without treatment, mean survival ranges from 2-4 years. Up to 3% of IPF patients have the familial form of the disease (familial pulmonary fibrosis or FPF). In 2001, autosomal dominant mutations in Surfactant Protein C (*SFTPC*) were shown to cause familial forms of lung disease, including FPF. Also, in 2006, mutations in genes encoding telomerase components, Telomerase Reverse Transcriptase (*TERT*) and an RNA component (*TERC*) were shown to increase susceptibility to FPF.

There is an unexpectedly high prevalence of FPF in Newfoundland. There are presently 14 identified FPF families with 54 affected individuals. The minimum prevalence of FPF in Newfoundland is 54 cases per 400,000, which is approximately 135 cases per 10<sup>6</sup>, compared to the FPF prevalence in the UK which is only 1.34 cases per 10<sup>6</sup>. Mutations in the *SFTPC* gene have previously been excluded in the FPF Newfoundland families. As well, mutations in the *TERT* and *TERC* genes have also been excluded in our 14 FPF families. In these families we are currently performing genome-wide scans to identify the causative gene(s) involved.

**P06.106****Association of Fc gamma receptors (FcγRs) polymorphisms with susceptibility of Systemic Lupus Erythematosus (SLE) in Hong Kong Chinese**

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Systemic Lupus erythematosus (SLE) is an autoimmune disease characterized by immune dysregulation, leading to high-level autoantibody production and immune complex deposition. Both genetic and environmental factors are known to contribute to the complex etiology of SLE. Fc gamma receptors, which constitute a clustered gene family located on chromosome 1q23, are low-affinity receptors for IgG and play important role in immune complex clearance. Abnormalities in FcγR mediated immune complex clearance could be involved in the disease and polymorphisms of FcγRs may affect the susceptibility and severity of SLE. In this study, we have examined SNP rs1801274 and SNP rs396991, two non-synonymous variations coding for amino acids located in the extracellular domain of FcγRIIa and FcγRIIIa, respectively. 430 SLE patients and 387 healthy controls of Hong Kong Chinese were genotyped for these two SNPs. The G allele of rs1801274 in FcγRIIa codes for R131, which was shown to have low-binding affinity to IgG. Our study found that the G/G genotype for this site was more frequent (14.2%) in SLE patients in our population compared to healthy controls (10.1%). No significant difference was seen in FcγRIIIa polymorphisms in Hong Kong Chinese. We are planning more extensive studies on this important locus and hypothesize that loss of balance among the receptors with opposing functions in this locus may confer disease susceptibility to SLE in Hong Kong Chinese.

**P06.107****Study of linkage to FEB1 and FEB2 loci in Serbian families with febrile seizures**

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Introduction: Febrile seizures (FS) represent the most common form of childhood seizure. It is well known that genetic factors play an important role in susceptibility to FS. Although specific genes that affect the majority of FS cases have not yet been identified, genetic loci for FS on chromosomes 8 (FEB1), 19 (FEB2), 2 (FEB3), 5 (FEB4), 6 (FEB5), and 18 (FEB6) have been reported recently. However, the mode of inheritance still remains unclear. Polygenic, autosomal dominant and autosomal recessive models have received support.

Aim: Our aim was to investigate the mode of inheritance in familial FS, to conduct the linkage analysis to FEB1 (8q13-21) and FEB2 (19p13.3) loci in Serbian population and to investigate the correlation between the specific locus and seizure type.

Methods: We investigated ten nonconsanguineous families with at least three members whose diagnosis of FS was established by the standard diagnostic criteria. The mode of inheritance was determined by pedigree analysis. In order to investigate the linkage of FS to FEB1 and FEB2 loci we analyzed microsatellite markers D8S1840, D8S530, D19S209, D19S216, D19S591. Alleles were determined after PCR and gel-electrophoresis and linkage was estimated by LOD score calculation.

Results: In ten investigated families we found the autosomal dominant mode of inheritance with reduced penetrance. Linkage analysis showed no significant correlation between febrile seizures and examined loci in our sample. Maximum LOD score of 0.98 at recombination fraction of θ=0.00 was obtained at locus D19S209. That confirmed previously presumed genetic heterogeneity of the disorder.

**P06.108****Familial Hemiplegic Migraine: linkage to chromosome 14q32 in a large Spanish kindred**

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<sup>4</sup>Corporació Sanitària Parc Taulí, Sabadell, Spain, <sup>5</sup>Departament de Genètica, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain, <sup>6</sup>CIBER Enfermedades Raras, Instituto de Salud Carlos III, Barcelona, Barcelona, Spain. Objective: To map the disease-causing gene in a large Spanish kindred with familial hemiplegic migraine (FHM), migraine with aura (MA) and migraine without aura (MO).

Methods: DNA samples from 20 family members were obtained. Patients were classified according to ICHD-II criteria for specific migraine subtypes. After ruling out linkage to known migraine genetic loci, a single nucleotide polymorphism (SNP)-based, 0.62 cM density genomewide scan was performed.

Results: In 13 affected subjects, FHM was the prevailing migraine phenotype in six, MA in four and MO in three. Linkage analysis revealed a disease locus in a 4.15 Mb region on 14q32, with a maximum two-point LOD score of 3.1 and a multipoint parametric LOD score of 3.8. This genomic region does not overlap with reported migraine loci on 14q21-22. Several candidate genes map to this region. Sequence analysis of one of them, *SLC2A4*, encoding a potassium-dependent sodium/calcium exchanger, failed to show disease-causing mutations in our patients.

Conclusions: The finding of a new genetic locus in FHM underscores its monogenic character and hints to greater genetic heterogeneity than previously suspected. While several genes conferring increased susceptibility to migraine seem to reside on 14q, the underlying disease-causing gene in our family remains unidentified.

#### P06.109

#### Association study of TOR1A gene polymorphisms with the risk of primary focal dystonia

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The most frequent cause of early-onset primary generalised dystonia, which is dominantly inherited, is a deletion of a GAG triplet from exon 5 of TOR1A gene (c.907delGAG). This gene encodes torsinA protein, whose role has been suggested to be involved in regulating nuclear envelope and endoplasmatic reticule organization. This 3-bp deletion removes a codon in the C-terminus of torsinA that normally encodes a glutamic acid residue, producing an altered protein, with reduced or no activity, which forms perinuclear inclusions.

A recent study described the effects of a new polymorphism in position c.646 (G>C) which causes the development of inclusions similar to those described for deltaGAG deletion. Moreover, this D216H amino-acid change reduced the number of torsinA inclusions in cultured cells with deltaGAG deletion. Many groups have studied the implication of this and other polymorphisms in this gene in primary dystonia but results are controversial. In this work we will try to find a risk haplotype for primary focal dystonia.

We analyzed 6 different polymorphisms in individuals with focal dystonia (n = 60) and a control healthy population (n = 50). None of them presented the GAG deletion in TOR1A exon 5. For genotyping, real-time PCR followed by allelic discrimination or PCR and automated sequencing was performed. Statistical analysis was carried out using a haplotype-based approach.

#### P06.110

#### Molecular diagnosis of Friedreich's ataxia in Macedonian patients

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Friedreich's ataxia (FRDA) is a progressive neurodegenerative disorder of autosomal recessive inheritance, in which gait ataxia followed by upper limb ataxia, dysarthria, nystagmus, areflexia, loss of joint position sense, and spastic paraparesis develop from the second decade of life. It is the commonest hereditary ataxia, with a prevalence of 1 in 50 000 and a deduced carrier frequency in European populations of 1 in 120. Friedreich's ataxia has been associated with mutations of the

frataxin gene on chromosome 9 (X25 at 9q13). In this paper we present our results from the molecular analysis of frataxin gene (X25) gene in total of 40 patients with spinocerebellar ataxia from the Republic of Macedonia. Fifteen of the patients have an early onset of progressive ataxia (before 25 years), while 25 patient were more than 25 years old at the time of diagnosis. We amplified the FRDA associated expanded fragment using a long range PCR technique. PCR product were analyzed by agarose and/or PAG electrophoresis. Mutation analysis shown that 14/15 patient with typical early onset of the symptoms were homozygous for a GAA expansion in intron 1 of frataxin gene. No expansion of the GAA repeat were found in 25 patient with ataxia more than 25 years old. In 35 normal individuals the number of GAA repeat were in normal range.

#### P06.111

#### Molecular characterization of two variants of GATA4 in patients with Congenital Heart Defects

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Congenital Heart Defects (CHDs) are among the most common developmental anomalies that affect around 1% of newborns. One of the genes described as causative of CHD is GATA4, a zinc finger transcription factor, important regulator in heart development. Mice lacking this gene have defects in the formation of heart tube and are lethal. GATA4 mutations have been found in families with Atrial and Ventricular Septal Defects and rarely associated with Tetralogy of Fallot.

In this study we report two different variants in the non coding sequence of GATA4 gene found in two patients with Atrial Septal Defect: c784-3 C>T and c998-4C>G. Interestingly, in one of the two cases neonatal permanent diabetes was present in the patient and her mother.

These variants were not detected in more than 100 healthy controls, while one of the two was also detected in the apparently patient's unaffected mother. Both patients were screened also for TBX5 and NKX2.5 genes and no mutations were found.

These GATA4 variants are located at acceptor splicing sites in intron 3 and 5, respectively, and predicted to be potentially able to affect splicing on the basis of specialized software analysis.

Since the gene is not expressed in lymphocytes and fibroblasts, the effect on splicing cannot be directly tested on cDNA. Therefore an in vitro assay to verify the effect of both nucleotide variants using an exon trapping model has been set up.

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#### P06.112

#### The distribution of S19W and -1131 T>C gene apolipoprotein A5 (ApoA5) polymorphism in children with innate risk factors of metabolic syndrome

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The risk factors of cardiovascular disorders there are in children and adolescence and sometimes may be cause of metabolic syndrome. One of metabolic syndrome criteria is atherogenic dyslipidemia with high level of triglyceride and low level of high density lipoprotein's cholesterol. The detecting of molecular markers of this dyslipidemia is one of the important problems.

The aim of our study was to investigate genotype and alleles distribution S19W and -1131 T>C ApoA5 gene polymorphism in children with risk factors of cardiovascular disorders and compare it with control group.

We included 187 children with risk factors of coronary artery disease (CAD) from 5 to 17 years old. Control group consisted of 150 children and adolescence with same age.

For detecting S19W and A-1131T>C ApoA5 gene polymorphism we used PCR with restriction assay by the method, described Talmud P. J. et al., 2002 .

We have revealed significant differences in genotype distribution (SS-90,4% and 81,9%, SW - 9,6% and 16,8%, WW - 0,0% and 1,3%, p=0,05, relatively) and alleles distribution (S-95,2% and 90,3%, W-

4,8% and 9,7%, relatively,  $p=0,025$ ) of S19W of *ApoA5* gene polymorphism between children with risk factors of CAD and control group. We haven't significant differences in genotypes and alleles distribution of A-1131T>C of *ApoA5* gene polymorphism in boys, girls and total group between children with risk factors of CAD and control group. **Conclusion:** children with risk factors of metabolic syndrome had more frequent SS genotype and S allele S19W of *ApoA5* gene polymorphism than in healthy children.

#### P06.113

#### Influence of TNF (G-308A) polymorphism on level of a TNF-alpha in blood at chronic diseases of lungs and liver

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Polymorphism in TNF (G-308A) gene was studied for association with tumor necrosis factor alpha level in blood of patients with chronic viral hepatitis ( $n=60$ ) and chronic obstructive pulmonary disease (COPD) ( $n=72$ ). The patients were from Tomsk population. It has been shown that level of TNF-alpha in patients with chronic viral hepatitis depends on TNF (G-308A) genotype: GG -  $137.9 \pm 222.49$ ; AG -  $48.7 \pm 58.16$ ; AA -  $31.0 \pm 1.41$  ( $p=0.018$ ). The finding suggests that in patients with chronic viral hepatitis allele A is associated with smaller intensity of an inflammation and fibrosis in a liver. In the patients with COPD, carriers of genotype AG had lower level TNF-alpha in blood at exacerbation ( $60.1 \pm 20.68$ ) and during remission ( $75.1 \pm 16.56$ ) in comparison with carriers of a genotype GG ( $193.8 \pm 50.65$ ;  $370.3 \pm 109.4$ ) ( $p=0.041$ ;  $p=0.026$ ). Genotype AA has not been revealed. Thus, at patients with COPD allele A is associated with low level TNF-alpha in blood, like in patients with chronic viral hepatitis. These results are discordant with known data on association of allele A with increased level of production of TNF-alpha. There might be several explanations of the discrepancy, including population specificity of genetic predisposition to various diseases and different LD structure in adjacent regions of genome (including 3'-UTR region of the gene) in different populations, as well as different environmental factors that can influence realization of a "genetic background".

#### P06.114

#### Somatic genome structural variations

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The notion that cells of common ancestry harbour genomes different from each other is strengthening. The differences may be either scheduled (e. g., immune response), or unscheduled (e.g., damage effects), and alter DNA structures. Simple repeats, interspersed sequences, (retro)transposons, pseudogenes, transgenes, etc, may assume unusual non-B conformations, affecting recognition by DNA-metabolizing enzymes. This may trigger a crescendo of variations, from base substitutions to aneuploidy. Epigenetics modifies chromatin and modulates transcription in somatic cells, but can be erased in germ cells. The resulting RNA may activate novel priming and retrotranscription thus concurring to genome rearrangements, competent to drive evolution and contribute to development. The resulting somatic genome structural variations (SGSV) may accelerate cell duplication and favour clonal amplification, occasionally optimizing some functions, but often deranging growth. Consequently, multicellular organisms are bound to be eventually genomic mosaic.

We report on: 1. the amplification of the genome of as few cells as possible of diverse somatic tissues, as a prerequisite for detecting SGSV; 2. their identification and characterization. For step 1, we have developed a modified isothermal whole genome strand displacement amplification based on the circularization of restricted genomes. For step 2, we are investigating two approaches, based on AFLP and differential 2D-gel electrophoresis; others are being considered such as mass sequencing and comparative genome hybridization. Benefits are expected in basic sciences (soma-germline-environment crosstalk, mechanisms of gene copy and chromosome number variations, evo-devo), as well as applications (diagnosis, therapy, stem cells, transgenetics, cloning, plant and livestock breeding).

#### P06.115

#### Cross-validation filtering for genome-wide association scan

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Genome-wide association studies (GWAS) with hundreds of thousands of markers are now feasible. In a one-stage study design, stringent significance thresholds are used because of the multiple-test problem. Hence, GWAs have low power to detect uncommon disease genes with low effects, even in relatively large datasets (several thousands of cases and controls). Alternative approaches have been proposed (Satagopan et al., 2002; Skol et al., 2005). Briefly, the full set of markers is tested in a fraction of the dataset only. A set of "best" markers, to be followed-up in either an independent (two-stages design) or an increased (joint-analysis design) sample, is identified from pointwise P-values. Yet, relaxing the significance thresholds may not compensate decrease in power due to the sample size reduction in stage-1. Here, we propose to use the consistency in the "associated" allele as a new criterion to select the "best" markers. The Consistency Rate (CR) is the number of times that allele 1 is found to be the risk allele out of n sub-samples. Each marker is ranked according to its CR value. Non-disease markers are expected to have CR values of 50%. Thus, in our approach, the "best" markers are those with CR values significantly different from 50%. We study the statistical properties of our filtering approach by simulations, using a panel of 30,000 SNPs from HapMap data, and under different conditions (disease gene effects; sample sizes; number of sub-samples n). We report type I error and power rates of our filtering and of other approaches.

#### P06.116

#### Identification of novel susceptibility loci 7q31 and 9p13 for bipolar disorder in an isolated population

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We performed a linkage analysis on 23 Finnish families with bipolar disorder and originating from the North-Eastern region of Finland, by using the Illumina Linkage Panel IV (6K) Array. The Panel IV had an average intermarker spacing of 0.64 cM across the entire genome. One phenotypic model, broad mood disorder including bipolar I disorder and recurrent depressive disorder, was used in the analyses. We found genome-wide significant evidence of linkage to chromosomes 7q31 (LOD = 3.20) and 9p13.1 (LOD = 4.02). Analyzing the best markers on the full set of 179 Finnish bipolar families supported the findings on chromosome 9p13 (maximum LOD score of 3.02 at position 383 Mb immediately upstream of the centromere). This region harbors several interesting candidate genes, including contactin associated protein-like 3 (CNTNAP3) and aldehyde dehydrogenase 1 (ALDH1B1). For the 7q31 locus, only one extended pedigree and seven families originating from the same late settlement region of Finland provided evidence of linkage, suggesting that a gene predisposing to bipolar disorder is enriched in that region of Finland. The loci on the centromeric region of 9p13 and telomeric region of 7q31 may represent novel susceptibility loci for mood disorder in the Finnish population.

#### P06.117

#### Mapping of a locus for Geroderma Osteodysplasticum to chromosome 1q23-q25

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Geroderma Osteodysplasticum (GO; OMIM%231070) is a rare autosomal recessive condition described so far in less than 30 patients whose molecular defect is still unknown. GO is characterized by pre-

mature aging, skin laxity and generalised osteoporosis with spontaneous fractures. Craniofacial dysmorphism include a prominent forehead, ptosis, large protruding ears, droopy cheeks, flat malar region and prognathism.

We ascertained a Lebanese family in which 3 individuals presented with GO. The proband aged 1 ½ years is born from sixth-degree consanguineous parents, while in his 2 maternal uncles (born from a maternal uncle who married his second-degree cousin) the diagnosis was posed at 21 and 18 years, respectively. A genome-wide search was performed by genotyping the 3 affected individuals for microsatellite markers spaced around 10-15 cM from the ABI PRISM® Linkage Mapping Set seeking for adjacent homozygous markers. Among 6 positive regions, haplotype reconstruction and genotyping of other family members allowed the identification of a unique informative 47 centimorgan region of homozygosity, shared only by the 3 patients. We generated 9 novel microsatellite markers in order to refine this region and outlined a locus for GO spanning from 155.15 to 174.71 megabases on the long arm of chromosome 1 (max Lod score 3.2). Sequencing of candidate genes within the linked interval is still in progress.

#### P06.118

##### Large deletions in 9q22.21-9q22.33 region of Gorlin syndrome patients

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Gorlin syndrome, also known as Nevoid Basal Cell Carcinoma Syndrome (NBCCS) or Basal Cell Nevus Syndrome (BCNS), is an autosomal dominant disorder that is characterized by various developmental abnormalities, like cysts of the skin, jaw cysts, bone malformations and different tumors like basal cell carcinomas (BCC) as most frequent, and also medulloblastomas, meningiomas, fibromas of the ovaries and heart. The prevalence is estimated at one per 57000. Phenotypical and genotypical diversity in Gorlin syndrome is referring to inactivating mutations in only one gene, PTCH, the human homologue of the *Drosophila* segment-polarity gene *patched*. PTCH is a tumor suppressor gene, located at 9q22.3, encoding a 12-pass transmembrane glycoprotein that acts as an antagonist in the Hedgehog signaling pathway. The 34 kb gene contains 23 exons and there are several hundred mutations, spanning the whole gene. Although there are some recurring mutations, there are no apparent hot spots. Nonsense, frameshift, in-frame deletions, splicesite, and missense mutations all have been described in the Gorlin syndrome patients. In some cases large deletions in PTCH region have been reported. We developed semi-quantitative fluorescent multiplex PCR with polymorphic markers surrounding PTCH and analyzed the whole 9q22 region in 50 Gorlin syndrome cases from France and Croatia. In three cases we found large deletions from 4-7 megabases in length, giving some depth and additional indications for phenotype diversity of the Gorlin syndrome.

#### P06.119

##### Analysis of CTLA4 Gene Polymorphisms in spanish Graves' Patients

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Graves' disease (GD) is a common organ-specific autoimmune disease that affects 0.5%-1% of Western population. It is a multifactorial disease that develops as the result of a complex interaction between genetic susceptibility genes and environmental factors. GD is characterized by diffuse goiter, ophthalmopathy, and anti-thyroid-stimulating hormone receptor antibodies.

Numerous studies have demonstrated the linkage to several polymorphism of the *CTLA4* gene. The *CTLA4* gene is placed on chromosome 2q33 and codifies the T cell receptor, which negatively modulates the immune response disabling the T cells. The aim of the present work was to determinate the contribution of C/T dimorphism in the promoter at position -318, the A/G dimorphism at position + 49 in exon 1, and the C/T60 dimorphism in the 3'UTR of the *CTLA4* gene to the severity

and manifestations of GD.

A cohort of 98 patients, and 50 healthy controls were used to genotype these three SNPs by PCR-RFLPs.

Results from +49A/G showed significant differences for the genotypes between Graves' patients and controls. The frequency of GG is higher than in controls, a 6% of the controls were homozygous for the G allele, while for the patients group we found 22%. Furthermore, significant increase in the frequency of the G allele was found in Graves' patients compared with controls ( $\chi^2 = 6.04$ ,  $P=0.0140$ )

No statistically significant association has been found with the -318 A/G and CT60 3'UTR polymorphism ( $\chi^2 = 17.8$ ,  $P = 0.1815$ ;  $\chi^2 = 0.79$ ,  $P=0.3729$  respectively).

#### P06.120

##### 5 base pair deletions in Rab27a gene as hot spot in exon 6 in Griscelli Syndrome (type II)

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Griscelli syndrome (GS), an autosomal recessive disorder, is characterized by a silver-gray sheen of the hair and the presence of large clusters of pigment in the hair shaft. GS can be associated to neurological impairment (GS1), immunodeficiency (GS2), or be isolated (GS3).

GS2 is caused by a mutation in the small GTPase **RAB27A** gene. The aim of this study was to investigate mutations in **RAB27A** gene, in a 3 year-old boy who was referred to our center with immunodeficiency and silver gray sheen of the hair.

Material and method: Genomic DNA was extracted from peripheral blood cells by salting out method. Five coding exons were amplified by PCR-Sequencing methods and sequenced to detect probable mutations in **RAB27A** gene.

Result: A homozygote deletion was found in exon 6 of the **RAB27A** gene.

Discussion: This deletion leads to a frame shift mutation in exon 6 and a premature stop codon. Clinical manifestations and immunodeficiency help physician to suspect diagnosis of GS II. As molecular analysis has provided an exact way to investigate mutations in immunodeficient patients like GS, it can be considered as a very useful method for definite diagnosis.

Because of an amino acid deletion and frame shift in this exon, we conclude that this new deletion is pathogen. Other deletions were found and reported in the same exon as causative mutation. We believe that this region may be a hot spot for GS II and suggest that the first analysis for other patients must be done in this region.

#### P06.121

##### Association study of the glycogen synthase kinase-3 beta (GSK3beta) gene with Mood Disorders

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Glycogen synthase kinase-3beta (GSK3beta) is a key component of the Wnt signaling pathway and is known to regulate such critical cellular functions as structure, gene expression, mobility and apoptosis. Recent findings support that GSK3beta may play a role in the pathophysiology and treatment of Mood Disorders (MD). We hypothesize that genetic variants, including SNPs and CNVs (Copy Number Variants)

tions) in the GSK3beta gene could partially underlie the susceptibility to mood disorders, just as much to unipolar major depression as to bipolar disorder. We performed a genetic case-control study including 440 screened control subjects and 445 patients with MD (257 Unipolar Major Depressive Disorder, 188 Bipolar Disorder). First, we selected a set of 11 TagSNPs polymorphisms representative of the patterns of common variation identified in European population in the genomic region containing the GSK3beta gene and genotyped them using SN-Plex Genotyping System. Moreover, we are replicating the experiment of Lachman et al. in our Spanish sample of MD patients. They found a statistical significant increased number of gains in a CNV partially overlapping GSK3beta in bipolar patients compared with control individuals. All the results were analyzed using SNPassoc package, from R software. Regarding TagSNPs analyses, nominal associations were found once stratified by polarity, even though they did not remain significant after correction for multiple comparisons. Overall, although genetic variants in GSK3beta gene might be a contributing factor to the development of mood disorders, further studies in larger samples are needed to obtain more conclusive results.

#### P06.122

#### Study of an association of deletion/insertion polymorphisms of *GSTM1* and *GSTT1* genes with a risk of 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. Endometriosis is regarded as one of the multifactorial diseases caused by an interaction between the environment and multiple genes.

Glutathione-S-transferases (GSTs) are enzymes involved in the phase II detoxification process of xenobiotics including carcinogens and mutagens. Gene polymorphisms of members of the GSTs family might impair detoxification function and increase the risk of the disorder. The present study was carried out to investigate if deletion/insertion polymorphisms of *GSTM1*, *GSTT1* and their combination are useful markers for predicting endometriosis susceptibility.

DNA was extracted from blood of 40 patients with reliable diagnosis of endometriosis and 50 healthy women (control group). DNA samples were amplified by polymerase chain reaction and detected by electrophoresis. The relative frequencies of the *GSTM1* wild/null and the *GSTT1* wild/null genotypes between both groups were compared.

The proportion of individuals with *GSTM1* null mutation was 45% in the endometriosis group and 30% in the control group ( $P > 0.05$ ), the proportions of *GSTT1* wild/null alleles in both groups were 22.5%/18% accordingly ( $P > 0.05$ ). The proportions of *GSTM1*(null/null) + *GSTT1*(null/null) genotype were 12.5%/2% ( $P > 0.05$ ). The distribution of the polymorphisms and their combination was not significantly different between the two groups. These results suggest that the *GSTM1* and the *GSTT1* null mutations aren't associated with a risk of the development of endometriosis in Russian women.

#### P06.123

#### Risk Factors of Inhibitors Development in Haemophilia Patients

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Introduction. FVIII inhibitors appearance in haemophiliacs is related to several factors, like substitution with native plasma products, frequent changing of FVIII concentrates, high rate of HBV and HCV infection, pseudotumours and other massive muscular haematomas, etc.

Objective. Starting from particularities of haemophilia treatment in our country, we evaluated the variables impact on inhibitors development. We assessed frequency and inhibitors titer, in correlation with risk factors.

Material and method: retrospective single center study based on 219 hemophilia patients registered and treated in the IIIrd Pediatric Clinic Timisoara, using Bethesda assay. We evaluated the influence of some parameters on frequency and inhibitors titer: haemophilia severity, gene mutations, family history, type of substitutive product used, hepatitis C or B infection, Interferon alpha therapy, surgical interventions,

and bolus vs. intravenous infusion of FVIII.

Results. Between January 1997- 2002, inhibitors frequency was 47.5%, whereas after January 2002 only 19.19% of patients had inhibitors due to reactants and methodology change. The majority of inhibitors were in severe haemophilia (78.95%), 15 of these patients being positive for intron 22 inversion.

Family history and exposure to FVIII were important risk factors of inhibitors development. Hepatitis C or B infection, Interferon alpha therapy, surgical interventions and intravenous infusion of FVIII haven't been significantly correlated with inhibitors' development.

Conclusions. In our patients, we found only the following risk factors for inhibitors development: exposure to FVIII, type of FVIII gene mutations and familial predisposition. Accuracy of technique and experience of laboratory technician is decisive for inhibitor evaluation.

#### P06.124

#### Association of the IL4R single-nucleotide polymorphism S411L with Hand Osteoarthritis

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Primary osteoarthritis (OA) is a common late-onset arthritis that demonstrates a complex mode of transmittance with both joint-site and gender-specific heterogeneity. In OA anti-inflammatory and anabolic cytokines which are usually responsible for the control of cartilage homeostasis are altered or inadequate. Hitherto, functional study had been mainly focused on the IL13/IL-4/IL-4R system which has a strong chondroprotective role and it is reasonable to speculate that polymorphism within these genes may be risk factors for OA. We therefore investigated polymorphisms in these genes as potential OA susceptibility loci by genotyping fifteen SNPs using TaqMan® Technology in 413 patients (26 male, 387 female) with hand OA and 326 healthy controls (14 male, 312 female). Although the majority of these SNPs had already shown an association with OA in previous studies, in our case-control study only one SNP (dbSNP rs1805013) was associated with  $P$ -values of 0.0125. This association was attributable to an increased frequency in the probands of the minor T allele regardless of gender and disease severity. This SNP results in the substitution of a serine with a leucine in the codon 411 of IL4R and consequently of an aliphatic hydrophobic residue for a polar hydrophilic one, a change that could have effects on protein function. Further functional studies for the S411L substitutions will clarify the role of this SNP on osteoarthritis predisposition.

#### P06.125

#### TMC1 but Not TMC2 Is Responsible for Autosomal Recessive Nonsyndromic Hearing Impairment in Tunisian Families

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Hereditary nonsyndromic hearing impairment (HI) is extremely heterogeneous. Mutations of the transmembrane channel-like gene 1 (*TMC1*) have been shown to cause autosomal dominant and recessive forms of nonsyndromic HI linked to the loci *DFNA36* and *DFNB7/B11*, respectively. *TMC1* is 1 member of a family of 8 genes encoding transmembrane proteins. In the mouse, *MmTmc1* and *MmTmc2* are both members of Tmc subfamily A and are highly and almost exclusively expressed in the cochlea. The restricted expression of *Tmc2* in the cochlea and its close phylogenetic relationship to *Tmc1* makes it a candidate gene for nonsyndromic HI. We analyzed 3 microsatellite markers linked to the *TMC1* and *TMC2* genes in 85 Tunisian families with autosomal recessive nonsyndromic HI and without mutations in the protein-coding region of the *GJB2* gene. Autozygosity by descent analysis of 2 markers bordering the *TMC2* gene allowed us to rule

out its association with deafness within these families. However, 5 families were found to segregate deafness with 3 different alleles of marker D9S1837, located within the first intron of the *TMC1* gene. By DNA sequencing of coding exons of *TMC1* in affected individuals, we identified 3 homozygous mutations, c.100C→T (p.R34X), c.1165C→T (p.R389X) and the novel mutation c.1764G→A (p.W588X). We additionally tested 60 unrelated deaf Tunisian individuals for the c.100C→T mutation. We detected this mutation in a homozygous state in 2 cases. This study confirms that mutations in the *TMC1* gene may be a common cause for autosomal recessive nonsyndromic HI.

#### P06.126

#### Genome wide association analysis in human height of European-originated monozygotic female twins

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Stature (i.e. adult height) is a quantitative trait with high heritability. Various interesting regions in the human genome have been linked to adult stature but only few have been confirmed by later studies. Two genome wide association (GWA) studies have been published recently and they identified two loci (HMGA2 and GDF5-UQCC) to be strongly associated to stature after extensive replication studies. In this study we report a genome wide association analysis performed on 1631 European monozygotic female twin pairs from the GenomEUtwin consortium. One of each pair was genotyped with the Illumina Human-Hap300-duo chip. Whole genome association analysis was performed with the PLINK-program. Area of residence and age were used as covariates in all of the analyses. Our study had two goals: We wanted to reduce the environmental variance by using the mean of each pair as a phenotype. We observed an association ( $p = 3.14 \times 10^{-6}$ ) on 8q24 locus underlying the linkage peak identified previously in our linkage scan on European dizygotic twins (LOD 3.28). The replication study is underway for the most significant findings in this GWA scan. Second, we analyzed whether we could pinpoint any regions in genome which would be associated to the difference within each pair. This approach was aiming to find genes responsible for increasing variance in human height, thus potentially indicating for example GxE interaction or imprinting/gene silencing. The most significant finding for variance in stature ( $p = 1.22 \times 10^{-5}$ ) was identified on 6q14 region.

#### P06.127

#### Hepcidin gene in adult HFE-negative hemochromatosis

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Hereditary Hemochromatosis (HH) is a disorder of iron homeostasis, characterized by increased iron absorption and tissue iron deposition, frequently related to HFE gene mutations. Hepcidin peptide (HAMP) down-regulates iron absorption; decreased HAMP expression is common in many HH forms and HAMP gene mutations are associated with juvenile HH.

Aim of this study was to investigate HAMP gene in 93 HH adult patients, negative for the most common HFE gene mutations, presenting >70% transferrin saturation.

Three exons and a promoter region of the gene were screened using DHPLC and sequencing. An additional upstream putative regulating region was also investigated.

The analysis did not show any classical mutations, but many previously undescribed variants were detected: 2 synonymous (L14L and T84T found in two patients), 5 variants in promoter and 5'UTR region, and one in the third intron. Interspecies comparison of HAMP sequences showed that 14 and 84 codons are highly conserved. The analysis of mRNA secondary structure, performed by MFOLD software, showed that the synonymous mutations here detected considerably modified the structure, particularly the 5' and 3'UTR regions. The novel promoter variant g.232C>T was found in 17% among patients and in 24%

among healthy controls ( $p>0.05$ ). The other promoter variants resulted sporadic in HH and absent in controls.

In conclusion, gene mutations causing defective HAMP peptide are not detected in our adult HFE-negative patients. However, HAMP variants might contribute to iron overload susceptibility, in addition to other genetic and/or environmental factors. Functional studies will be necessary to test this hypothesis.

#### P06.128

#### CSCE-based mutation analysis of *NIPA1* gene revealed no mutations in Italian patients with autosomal dominant hereditary spastic paraplegia

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We set up a new method based on Conformation Sensitive Capillary Electrophoresis (CSCE) to screen a cohort of 62 Italian families with autosomal dominant hereditary spastic paraplegia (ADHSP) for mutations in *NIPA1* gene (SPG6). Probands of all families were previously excluded for known mutations in the *SPG4* gene, *SPG3A*, *SPG13* and *SPG31* genes. CSCE adapts the technique of heteroduplex analysis on automated capillary array electrophoresis using the Applied Biosystems 3130 Genetic Analyzer. A previously published *SPG6* mutation in a Japanese ADHSP patient (Kaneko et al., 2006) was used as a positive control in the optimization of the analysis protocol. We analyzed the five coding exons and flanking intronic sequences of *NIPA1* gene and we didn't find any mutation in our cohort of patients. Up to now, only four different nucleotide changes have been reported in the *NIPA1* gene in populations from different geographic areas, two of them leading to the same aminoacidic change (G106R). Our negative result shows that *SPG6*-linked form is a rare cause of ADHSP in the Italian people, in confirmation of another study performed on European ADHSP families (Klebe et al., 2007). In this study, we provide a rapid, sensitive and effective protocol of automated mutation detection, also applicable to large genes and to high-throughput screening.

#### P06.129

#### HIF1A gene polymorphism is associated with power performance in athletes

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Glycolysis is the central source of anaerobic energy in humans, and this metabolic pathway is regulated under low-oxygen conditions by the transcription factor hypoxia-inducible factor 1a (HIF-1a). HIF-1a controls a number of genes that are implicated in various cellular functions including cell proliferation (erythropoietin), glucose metabolism (glucose transporters and glycolytic enzymes), cell survival, and angiogenesis (vascular endothelial growth factor and VEGF receptors). A missense polymorphism, Pro582Ser, is present in exon 12 (C/T at bp 85). The rare T-allele is predicted to result in a proline to serine change in the amino acid sequence of the protein. This substitution increases protein stability and transcriptional activity, and therefore, improves glucose metabolism and angiogenesis. In this study, we investigated whether genetic variation at the locus encoding HIF1A is associated with elite athlete status in weightlifters, for which glycolysis is crucial for power performance. The study involved 53 Russian athletes (17 sub-elite, 32 elite and 4 highly elite) and 920 controls. HIF1A gene Pro582Ser polymorphism was determined by PCR-RLFP. The frequency of the rare Ser allele was significantly higher in weightlifters than in controls (17.9% vs. 8.5%;  $P=0.001$ ). Moreover, the frequency of Ser allele increased with growing skill level of athletes (sub-elite (14.7%) - elite (18.8%) - highly elite (25.0%)). Thus, HIF1A gene Pro582Ser polymorphism is associated with elite power athlete status, which suggests an important role for HIF-1a in skeletal muscle adaptation to power training.

**P06.130****Association of the *RET* proto-oncogene to Intestinal Neuronal Dysplasia type B**

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<sup>1</sup>Unidad de Gestión Clínica de Genética, Reproducción y Medicina Fetal, Hospitales Universitarios Virgen del Rocío, Seville, Spain, <sup>2</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Seville, Spain. Hirschsprung disease (HSCR) is defined by the absence of intramural ganglia of Meissner and Auerbach along variable lengths of the gastrointestinal tract. Intestinal neuronal dysplasia type B (INDB) is characterized by the malformation of the parasympathetic submucous plexus of the gut. It seems to exist a connection between these two enteric nervous system abnormalities, supported by the fact that HSCR often includes a region of INDB-like character proximal to the aganglionic segment. Because of the major role played by the *RET* proto-oncogene in HSCR, we sought to determine if this gene was also related to INDB, by both a mutational screening in a series of patients, and the evaluation of its polymorphisms/haplotypes as susceptibility factors for this disease. We have used dHPLC techniques to screen the *RET* coding region in 23 patients presenting with INDB, and 30 patients with a combined HSCR+INDB phenotype. In addition, a total of 11 *RET* SNPs were strategically selected and genotyped by using Taqman technology. Distribution of such SNPs, as well as of the haplotypes generated by their combinations, was compared among the different groups of patients (INDB, HSCR+INDB, HSCR) and controls. As a result we have demonstrated the involvement of *RET* in the pathogenesis of intestinal neuronal dysplasia B, although by different molecular mechanisms to those leading to Hirschsprung disease. Further investigation is warranted in order to elucidate those precise mechanisms and to clarify therefore the genetic nature of INDB.

**P06.131****First Dutch founder mutation in Hereditary Spastic Paraplegia**

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Hereditary spastic paraplegia (HSP) is characterized by clinical and molecular heterogeneity. In autosomal dominant (AD) spastic paraplegia (SPG), *SPASTIN* (SPG4) and *ATLASTIN* (SPG3A) gene defects account for approximately 40% and 10%, respectively.

We performed parametric linkage analysis, using the Affymetrix 10K SNP array, to identify the SPG locus in a ten-generation Dutch pedigree. A maximum LOD score of 5.03 was obtained at the SPG31 locus (2p11-p12). Mutation analysis of the receptor expression-enhancing protein 1 gene (*REEP1*) was performed in 10 additional AD SPG families from the South-East part of the Netherlands. A truncating four basepair deletion in exon six (c.537\_540delCGGC p.Ser179ArgfsX43) was identified which co-segregated with the disorder in the large linked family and in two other small unrelated families, suggesting a founder effect in our region. A founder effect was confirmed by haplotype analysis using polymorphic markers surrounding *REEP1*. The clinical features within these families ranged from normal to severe spasticity of legs and the age of onset varied from birth till >75 years of age. There was an inverse correlation between age at onset and severity and progression of symptoms. Further functional studies are needed to identify a major modifier in *REEP1* affected families.

In conclusion, we identified a founder *REEP1* mutation in 27% (3/11) of the AD pure SPG families investigated in the South-East part of the Netherlands. Thus *REEP1* gene defects in HSP seem at least as common as *ATLASTIN* (SPG3A).

**P06.132****Quantitative trait locus mapping in complex human pedigrees with interpopulation origin**

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We perfected the variance-components method for quantitative trait locus (QTL) analysis of complex human pedigrees descended from interpopulation crosses between outbred parental lines with different

QTL allele frequencies in each population. Furthermore, dominance and inbreeding are allowed for. The updated method is based on the decomposition of trait variance into components with the consideration of the genetic effect conditioned by interpopulation origin and inbreeding of individuals. To estimate model parameters, namely additive and dominant effects, and the allelic frequencies of the QTL analysed, and also to define the QTL positions on a chromosome with respect to genotyped markers, we used the maximum-likelihood method. To detect linkage between the QTL and the markers we propose statistics with a noncentral chi-square distribution that provides the possibility to deduce analytical expressions for the power of the method and therefore, to estimate the pedigree's size required for 80% power. The method works for arbitrarily structured pedigrees and uses the phenotypic values and the marker information for each individual of the pedigree under observation as initial data and can be valuable for fine mapping purposes. The power of the method is increased if the QTL effects conditioned by interpopulation origin and inbreeding are enhanced.

**P06.133****Clinical and genetic investigation of three kindreds with familial hyperparathyroidism**

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We investigated three Italian kindreds referred to our Surgery Unit for familial hyperparathyroidism. One kindred was diagnosed as affected by hyperparathyroidism-jaw tumor syndrome (HPT-JT), due to the occurrence of a maxillary ossifying fibroma, the other two kindreds were defined as familial isolated hyperparathyroidism (FIHP) because of the occurrence of hyperparathyroidism without other syndromic manifestations. Clinical investigation demonstrated that all kindreds shared other clinical features besides hyperparathyroidism, i.e., high frequency of uterine polypsis and thyroid neoplasms. Since germline *HRPT2* mutations are detected in HPT-JT kindreds, but also in 7% of FIHP kindreds, we tested patients for *HRPT2* mutations and performed immunohistochemical analysis of parafibromin, encoded by *HRPT2*, in all available tumor tissues. Germline *HRPT2* inactivating mutations were identified in the HPT-JT kindred and in both FIHP kindreds. A *HRPT2* somatic mutation was also demonstrated in a parathyroid adenoma from a FIHP patient, in agreement with *HRPT2* tumor-suppressor role. Moreover, loss of nuclear parafibromin expression was demonstrated in all parathyroid tumors, at variance with findings in biopsies from normal parathyroid glands. In addition, immunohistochemistry performed on a HPT-JT-related uterine polyp did not show any nuclear anti-parafibromin reactivity, as compared with five sporadic polyps in which almost all stromal cells exhibited nuclear parafibromin immunostaining. Overall, our results indicate that FIHP and HPT-JT associated with *HRPT2* mutations do not have a distinct genetic signature, but may represent variants of the same genetic disease. Loss of parafibromin expression in polyps supports the pathogenetic role for parafibromin in uterine polypsis associated with this syndrome.

**P06.134****Investigation the role of -344C>T polymorphism in CYP11B2 gene among Bulgarian hypertensive patients**

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**Background:** The renin-angiotensin-aldosterone system is often investigated in relation to the pathogenesis of essential hypertension. The mineralocorticoid hormone aldosterone plays an important role in blood pressure homeostasis. A key factor for its synthesis is the enzyme aldosterone synthase, encoded by CYP11B2 gene. The -344C>T polymorphism in the 5' regulatory region of the CYP11B2, which disrupts a putative binding site for the steroidogenic factor 1, was reported to be associated with aldosterone excess and hypertension.

**Objective:** Our aim was to investigate the role of functional -344C>T polymorphism in the promoter region of CYP11B2 gene in the manifestation of hypertension, using case-control study design.

**Methods:** We included 185 Bulgarian hypertensive patients and normotensive control group. The -344C>T polymorphism was genotyped by RCR-RFLP method using HaeIII restrictionase.

**Results:** The genotype and allele frequencies for -344C>T variant in

our normotensive control group were similar to those detected in other Caucasian populations. The genotype distribution of this polymorphism did not show statistically significant differences between the two explored groups ( $p=0.822$ ). The comparison of the groups, divided by gender, also did not demonstrate significant differences in the genotype and allele distribution ( $p>0.05$ ).

**Conclusion:** Our study does not support the involvement of the -344C>T polymorphism of CYP11B2 gene in the susceptibility to hypertension in Bulgarian patients. Due the small sample size, however, further studies in larger groups are warranted.

#### P06.135

##### Increased frequency of the IVS4+39C>T allele of PGHD-15 gene in group of patients with severe course of IBD

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Inflammatory bowel diseases (IBD) are autoimmunological disorders (MIM #266600) with genetic background, characterized by chronic inflammation of the wall of gastrointestinal tract. IBD include two clinical entities: Crohn's disease and ulcerative colitis (MIM#191390). The incidence of these diseases in Western population ranges from 100 to 300 per 100 000. The symptoms of Crohn's disease may arise in any part of gastrointestinal tract, however most often the distal portion of the ileum and caecum is affected. Inflammatory process penetrates through the whole thickness of the bowel wall and skip lesions are typical. Continuous inflammatory lesions confined only to colonic and rectal mucosa are characteristic for ulcerative colitis. Fistulas are absent and the inflammation never adopts the granulomatous form.

In our research we examined the frequency of alleles in the NOD2 gene and 15-@hydroxyprostaglandin dehydrogenase gene (PGDH-15) in 58 patients with severe postoperative relapses of inflammatory bowel diseases, 27 children with inflammatory bowel diseases and in control group (100 persons). The average age of onset was 31 years in the group of patients with severe disease course and 11 in the group of children. We observed the elevated frequency of the alleles 3019-3020insC and 802C>T of the NOD2 gene in comparison to the control group. The allele IVS4+39C>T of the PGDH-15 gene in the group of ill with the heavy course occurred two times more frequent than in the control group and three times more frequent in comparison with the group of affected children.

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#### P06.136

##### MMP1 G-1607GG and MMP9 C-1562T gene variants in idiopathic bronchiectasis

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Bronchiectasis is a chronic infective and inflammatory disease characterized by dilatation of bronchi and sputum production. Although the pathogenesis of bronchiectasis is not fully understood, destruction of the bronchial wall that contributes to the development of airway dilatation may result from excessive extracellular matrix degradation. Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, which play an essential role in tissue remodeling and repair. Genetic variants of genes involved in extracellular matrix remodeling may drive the pathological processes that could lead to the development of bronchiectasis. The aim of this study was to analyse MMP1 G-1607GG and MMP9 C-1562T promotor polymorphisms in development of idiopathic bronchiectasis.

The genotypes of 37 patients with idiopathic bronchiectasis and 102 controls were determined by conformation sensitive gel electrophoresis for MMP1 G-1607GG gene variant and PCR-RFLP for MMP9 C-1562T polymorphism.

The distribution of -1607GG allele was significantly higher in patient group ( $p=0.014$ ). Heterozygote carriers of -1607GG allele had a 5.3-

fold increased risk for bronchiectasis development (62.2% vs. 50%,  $p=0.013$ ). The OR was even greater (8.7) for homozygous -1607GG genotype (29.7% vs. 18.6%,  $p=0.006$ ) compared with homozygotes for 1G allele.

The MMP9 C-1562T allelic and genotype frequencies did not show significant differences between the groups.

According to our results MMP1 -1607GG variant might be involved in pathogenesis of idiopathic bronchiectasis. This polymorphism is associated with higher gene expression and may influence lung parenchyma damage and subsequent bronchial wall destruction and development of airway dilatation.

#### P06.137

##### Novel differentiating genetic markers for CD and UC

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CD and UC belong to the inflammatory bowel diseases (IBD) with heterogeneous clinical manifestations, genetic factors and response to treatment. That provides increased interest for detection of differentiating factors valuable for precise early diagnostics of these diseases.

Five polymorphic variants of two genes (*Gly908Arg*, *Arg702Trp*, *Leu1007fins* of *NOD2/CARD15* gene and -308G/A, -238G/A of *TNFA* gene) were studied in CD patients (n=102), in UC patients (n=71) and also in control group (n=100). The frequency of -308A allele of the *TNFA* gene was increased in both groups of patients with CD and UC as compared to controls (15%, 11% and 4%, respectively). We have found an extremely significant difference for *Leu1007fins*, mutation of *NOD2/CARD15* gene which results in truncated protein. This mutation was revealed in 15% of patients with CD and only in 2% of patients with UC ( $p=0.0055$ ). In control group the frequency of this mutation was 3%. The frequencies of carriers of two others genetic variants (*Gly908Arg*, *Arg702Trp*) were lower in UC patients (4% and 1%) as compared to these ones in CD patients (8% and 6%) but it was not significant.

Thus that mutation *Leu1007fins* of gene *NOD2/CARD15* plays a crucial role in pathogenesis of Crohn's disease and might be treated as a differentiation marker between patients with CD and UC. Consequently that may improve diagnostics of these diseases and provide appropriate treatment.

#### P06.138

##### Associations of CARD15, DLG5, OCTN1, TLR4, IL1, IL10 and TNFa genes variants with Inflammatory Bowel Disease

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Inflammatory bowel disease (IBD) is a multifactorial polygenic disease with probable genetic heterogeneity. It is characterized by chronic inflammation of the gastrointestinal tract.

64 unrelated patients from 6 to 14 years old with IBD and 52 controls children were investigated. The aim of our research was searching association between IBD and nine polymorphous variants of seven genes. The analysis for *CARD15* R702W, G908R, Leu1007fs, *DLG5* R30Q, *OCTN1* L530F, *TLR4* D2999G, *IL10* c.-1082 g>a, *TNFa* c.-308 g>a variants, and *IL1* VNTR polymorphism was performed by means of MLPA and AFLP analysis.

Results of genotyping are presenting in the table.

Thus it is not revealed associations between carriage of the variant alleles these genes and Inflammatory Bowel Disease at Russian children.

Allele frequencies of the minor alleles (%)

gene	<i>CARD15</i>	<i>CARD15</i>	<i>CARD15</i>	<i>DLG5</i>	<i>OCTN1</i>	<i>TLR4</i>	<i>IL1</i>	<i>IL10</i>	<i>TNFa</i>
allele	702W	908R	c.3020 insC	30Q	503F	299G	574	c.-1082a	c.-308a
IBD group	2,3	3,9	5,5	5,5	46,7	15,3	4,7	0,8	7,0
Control group	2,9	2,9	4,0	5,9	39,6	11,7	0	0	9,7
Fisher exact test (P)	1,00	1,00	0,76	1,00	0,43	0,65	0,16	1,00	0,57

**P06.139****CARD15, TNF-alpha, IL23R and ATG16L1 genes polymorphisms in Lithuanian patients with inflammatory bowel disease**

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**Background.** Numerous genome-wide and linkage studies have identified and replicated significant association between inflammatory bowel disease (IBD) development and polymorphisms of genes attributed to recognition of bacterial products (CARD15), innate immune responses (TNF-alpha, IL23R), and autophagosome pathway (ATG16L1). Despite large number of studies accumulated in this field, there is only limited data on IBD genetic characteristics in East and Central Europe region.

**Aim and Methods.** This research aimed to analyze the frequency of the three CARD15 polymorphisms Arg702Trp (rs2066844), Gly908Arg (rs2066845) and Leu1007finsC (rs2066847); two TNF-alpha promoter polymorphisms -857C>T (rs1799724) and -308G>A (rs1800629); IL23R polymorphism Arg381Gln (rs11209026); and ATG16L1 polymorphism Thr300Ala (rs2241880) and their contribution to the development of IBD in a cohort of unrelated Crohn's disease (CD) (n=57), ulcerative colitis (UC) (n=123) patients and healthy controls (n=182) from the Lithuanian population.

**Results.** Carriage of CARD15 variants was more common in the CD patients than in controls and UC patients (40.4% CD versus 17.6% controls and 16.3% UC, p<0.0001). The single marker analysis of the CARD15 mutations revealed a strong association between Leu1007finsC rare variant and CD (p=3.687x10-8; OR=5.54, 95% CI: 2.85-10.75). No genetic association was detected between other two CARD15 variants (Arg702Trp and Gly908Arg), TNF-alpha promoter, IL23R and ATG16L1 polymorphisms and IBD in Lithuanian population.

**Conclusion.** Our study has revealed a strong association only between CARD15 gene variant Leu1007finsC and CD susceptibility in the Lithuanian population.

**P06.140****Understanding the role of IRAK-M in allergic asthma: replication in European children and functional analyses**

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Asthma is a multifactorial disease influenced by genetic and environmental factors. Interest in finding etiologic factors has recently intensified, because of its increasing prevalence and associated mortality. Analysing a cohort of allergic asthmatics in the Sardinian founder population, where limited genetic and environmental heterogeneity facilitates the study of multifactorial traits, we recently identified IRAK-M as a new asthma susceptibility gene and replicated the association in a genetically distant Italian population. IRAK-M, a negative regulator of the TLR/IL-1R pathways and a master regulator of NF-κB and inflammation, is associated with early-onset persistent asthma and highly expressed in lung epithelia. Thus, our data suggest a link between hyperactivation of the innate immune system and chronic airway inflammation, indicating IRAK-M as a new target for therapeutic intervention against asthma.

To better understand the role of IRAK-M in the pathogenesis of asthma, we are carrying out a replication study in two European cohorts (the PARSIFAL and the BAMSE study groups), including children affected by asthma and/or its sub-phenotypes. In these samples we are evaluating the association of 6 SNPs defining the IRAK-M risk haplotype as well as gene-gene and gene-environment interactions in the predisposition to asthma. In parallel, we are analysing relevant IRAK-M variants identified by mutation screening in 314 Sardinians affected by allergic asthma and 200 controls. Experiments to evaluate the functional effect of the mutations on NF-κB activity and inflammation in monocyte- and

pneumocyte-derived cell lines as well as the regulation of IRAK-M expression are in progress.

**P06.141****Genetic polymorphisms of the insulin receptor substrate-1 (IRS-1) gene and profiles of clopidogrel-induced antiplatelet effects in type 2 diabetes mellitus patients with coronary artery disease**

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<sup>1</sup>Department of Mother and Child and of Biology-Genetics, Section of Biology-Genetics, University of Verona, Verona, Italy, <sup>2</sup>Division of Cardiology, University of Florida College of Medicine-Shands Jacksonville, Jacksonville, FL, United States, <sup>3</sup>Cardiovascular Institute, San Carlos University Hospital, Madrid, Spain. **Background:** Patients with T2DM have reduced responsiveness to the platelet P2Y<sub>12</sub> receptor antagonist clopidogrel compared to non-diabetics. However, variability in clopidogrel-induced antiplatelet effects has been observed among T2DM and those with elevated platelet reactivity have a higher risk of atherothrombotic events. Since variation in insulin sensitivity modulates platelet P2Y<sub>12</sub> signaling, the aim of this study was to evaluate if SNPs of the insulin receptor substrate-1 (IRS-1) are associated with variation in the response to clopidogrel in T2DM patients.

**Methods:** A total of 7 tagSNPs (rs1801278, rs11683087, rs1801123, rs1896832, rs956115, rs2251692, rs6725330) were determined in 180 T2DM patients with coronary artery disease in a steady state phase of clopidogrel therapy. Patients were classified into carriers and non-carriers of the variant allele for each SNP according to a dominant model. In addition, haplotype distribution of IRS-1 genes within our population was assessed. Platelet function was determined by LTA following 20 μM adenosine diphosphate stimuli (ADP). Platelet aggregation according to genotype and haplotype was determined.

**Results:** The 7 tagSNPs were accountable for 93% of the haplotype distribution of the IRS-1 gene. Platelet aggregation was 55±15% in the overall population. Preliminary results showed an association between carriers of the variant C allele of the rs956115 SNP (n=34) and the highest degree of platelet reactivity (60±14%; p<0.05).

**Conclusions:** rs956115 polymorphism of IRS-1 gene is associated with lower clopidogrel-induced antiplatelet effects in T2DM patients with coronary artery disease.

**P06.142****Polymorphism of insulin receptor substrate-2 gene in Turkish with type 2 diabetes mellitus**

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Insulin receptor substrate-2 (IRS-2) is an endogenous substrate for the insulin receptor tyrosine kinase, which play a key role in insulin signaling. IRS-2 gene is potential candidate for development of type 2 diabetes mellitus. In this study, we have identified IRS-2 gene polymorphisms, evaluated their frequencies in Turkish subjects, and analyzed the contribution of these polymorphisms to the development of type 2 diabetes mellitus.

Study subjects were recruited from patients of Endocrinology Department, Selcuk University. We chose 122 unrelated, non-obese patients with type 2 diabetes mellitus and 35 unrelated, non-diabetic subjects with no family history of diabetes as age and body mass index matched control subjects. The coding and non-coding sequence of IRS-2 gene (divided into 15 overlapping fragments) was amplified by polymerase chain reaction and screened for the presence of single stranded conformational polymorphisms (SSCP).

Five missense (Thr<sup>849</sup>→Ser, Arg<sup>1304</sup>→Gly, Lys<sup>1253</sup>→Asn, Cys<sup>1318</sup>→Arg, Gly<sup>1057</sup>→Asp) and six silent mutations (CCT→CCC encoding Pro<sup>483</sup>, GCT→GCC encoding Ala<sup>488</sup>, GGT→GGC encoding Gly<sup>497</sup>, CCC→CCT encoding Pro<sup>829</sup>, ACC→ACG encoding Thr<sup>845</sup>, TGT→TGC encoding Cys<sup>807</sup>) were detected in IRS-2 gene. The Thr<sup>849</sup>→Ser (6/122) and Gly<sup>1057</sup>→Asp (8/122) mutations were more common in diabetic patients for these variants. Four novel amino acid variants were described and identified in the coding region of the IRS-2 gene. There was no significant association found with these variants and diabetes.

As a conclusion, IRS-2 Arg<sup>1304</sup>→Gly, Lys<sup>1253</sup>→Asn and Cys<sup>1318</sup>→Arg mutations rare and IRS-2 Thr<sup>849</sup>→Ser and Gly<sup>1057</sup>→Asp mutations were

common in our population. But may not be major determinants in genetic susceptibility to type 2 diabetes.

#### P06.143

#### Association study of homocysteine metabolic regulatory genes with ischemic stroke risk in Singapore Chinese population

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Moderately elevated plasma homocysteine level has been identified as an independent risk factor for vascular disease, including ischemic stroke. There are also substantial evidences for homocysteinaemia to be, at least partially, influenced by genetic factors. The focus of this study is to investigate the association between the 25 candidate genes of the homocysteine metabolic pathway and ischemic stroke risk in Chinese population. We genotyped 306 polymorphic SNPs from the 25 candidate genes in 376 stroke patients and 354 matched healthy controls from Singapore. Using genotype-based trend test, we identified 3 novel polymorphic loci from 3 genes (MTRR, SHMT1 and TCN2) which act as potential genetic risk factors for ischemic stroke. The effects of these loci are independent from each other as well as other known risk factors including age, gender, smoking, diabetes, hypertension and hyperlipidemia. We also found that the association of TCN2 variants with stroke appears to be more predominant in patients with lacunar infarction (LACI) than in other stroke subtype (non-LACI). In summary, the variants of these genes potentially affect individual susceptibility to ischemic stroke through the homocysteine metabolic pathway. Here, through genetic association study, our result supports the importance of B12 and folate cellular availability to prevent ischemic stroke in Chinese population. The growing evidence of importance of folate and B12 has significant implications for the inclusion of folic acid and B12 as supplement to prevent stroke. To our knowledge, this is the first comprehensive analysis of the homocysteine metabolic pathway in stroke.

#### P06.144

#### Prevalence and phenotypic spectrum of known genes causative of Joubert Syndrome and Related Disorders: the experience of the International JSRD Study Group

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Joubert syndrome is an autosomal recessive condition characterized by hypotonia, ataxia, psychomotor delay, oculomotor apraxia, neonatal breathing abnormalities, and a complex midbrain-hindbrain malformation known as the "molar tooth sign" (MTS). The variable multiorgan involvement (mainly the retina and kidneys) defines several pleiotropic conditions (Joubert Syndrome Related Disorders, JSRDs) sharing the MTS. The nosological definition of JSRDs has long remained problematical, and a recently proposed classification is now based on the extent of retinal and renal involvement. Two loci (JBTS1-2) and five genes (NPHP1, AHI1, CEP290, MKS3, RPGRIP1L) have been identified so far. All genes encode for ciliary proteins, and JSRDs present clinical and genetic overlap with other ciliopathies, such as nephronophthisis and Meckel syndrome.

Over the past 3 years, on behalf of the International JSRD Study Group, we have performed mutation screening of known genes in large cohorts of about 100-150 patients representative of all JSRD phenotypes. These allowed estimating the prevalence of gene mutations in different subgroups and drawing preliminary gene-phenotype correlates. Pure JS is mostly caused by AHI1 mutations (~10% of cases), and occasionally by MKS3. Cerebello-retinal phenotypes

are also mainly related to AHI1 (~20% of cases) although CEP290 can be rarely responsible. The genes most frequently causative of cerebello-renal phenotypes are NPHP1 and RPGRIP1L (~10% of cases each). Up to 50% of cerebello-oculo-renal cases are due to mutations in CEP290, while a minority relates to NPHP1 deletions. Overall, mutations in known genes are responsible of only 25-30% of JSRDs, supporting further genetic heterogeneity.

#### P06.145

#### Kir 4.1-KCNJ10 potassium channel gene polymorphisms in Type 2 diabetes

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Potassium channels play an important role in insulin secretion. KCNJ10 channel protein is a member of Kir 4.1 potassium channel family. KCNJ10 gene is expressed in pancreatic beta cells. We therefore investigated whether polymorphisms in KCNJ10 gene are associated with type 2 diabetes in Turkish population.

In this study 166 individuals with type 2 diabetes and 100 age-and sex-matched healthy controls were tested for three single nucleotide polymorphisms (SNPs) in KCNJ10 gene. These SNPs were G to A transversion in intron 1 (SNP1) and G to A transversion in exon 2 (SNP2) and T to C transition in promoter (SNP3). All SNPs were genotyped by PCR-RFLP.

KCNJ10 gene SNP1 in intron 1 and SNP2 in exon 2 which were non-informative for Turkish population. The distribution of TT, TC, and CC genotypes for SNP3 in promoter was 56 %, 37% and 5 % in type 2 diabetes compared with 45 %, 51 % and 4 % in the controls ( $\chi^2=4.352$ ,  $p=0.113$ ). The allele frequency of T and C was 0.705, 0.295 in type 2 diabetes compared with 0.756, 0.244 in the controls ( $\chi^2=1.676$ ,  $p=0.195$ ). According to the "Hardy-Weinberg Equilibrium" TT genotype was found higher in type 2 diabetes than TC genotype ( $\chi^2=4.07$ ,  $p=0.04$ ).

We conclude that KCNJ10 gene SNPs were not associated with type 2 diabetes in Turkish population. However, further studies with larger samples are needed to address the exact role of KCNJ10 gene in type 2 diabetes and its complications such as diabetic retinopathy.

#### P06.146

#### Association of the CALM1 core promoter polymorphism with knee osteoarthritis in patients of Greek origin

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Osteoarthritis (OA) is characterized by focal areas of loss of articular cartilage in synovial joints, associated with varying degrees of osteophyte formation, subchondral bone change and synovitis. Calmodulin-1 gene (CALM1) encodes a ubiquitous eukaryotic  $\text{Ca}^{2+}$  binding protein and is the principal mediator of the calcium signal thus affecting the chondrocyte's response to mechanical load. A functional core promoter SNP -16C/T (rs12885713) was recently associated with HOA in the Japanese but not the British patients. In multifactorial genetic traits, each gene contributes to disease susceptibility to some extent, but such genes may also interact with each other. The aspartic-acid D14 repeat allele of the asporin gene (ASPN) is a susceptibility allele for both HOA and KOH in the Japanese and Caucasians. Our case-control study (158 KOA and 193 controls) focuses on genotyping Greek KOA patients for rs12885713, and investigating its role as a risk factor for the development of KOA. Our previous work on the same case-control group indicated that both the D14 and D15 alleles increased risk for KOA therefore we additionally investigate whether a combined effect of the ASPN D14/D15 alleles and CALM1 TT exists in Greek KOA patients. No significant differences were found in genotype frequencies for the -16T/C SNP of CALM1 gene between cases and controls ( $p=0.581$ ). Our data implied that the -16TT (rs12885713) CALM1 core promoter genotype is not a risk factor for KOA etiology in Greek Caucasians on its own but associated with the ASPN D14 or D15 risk allele it could influence KOA susceptibility.

**P06.147****A haplotype of the lipopolysaccharide-binding protein gene is associated with susceptibility to severe sepsis**

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The lipopolysaccharide-binding protein (LBP) is an acute phase protein that plays a dominant role in the genesis of sepsis by initiating signal transduction pathways leading to the activation of the inflammatory host response. A single nucleotide polymorphism (SNP) of the *LBP* gene has been previously associated with an increased risk for sepsis but gene-wide association studies are lacking. Here we evaluated the association of common variants across the entire *LBP* gene with susceptibility to severe sepsis by genotyping 10 selected SNPs, including a subset of tagging SNPs that efficiently captured common variation across the gene, in 176 severe cases of sepsis and 364 population-based controls. Although individual SNPs resulted non-significantly associated after adjusting for multiple testing, a haplotype extending ~1.2 kb in the 5'-flanking region of *LBP* gene, crucial for *in vitro* gene inducibility by cytokines, showed a significant association with susceptibility to severe sepsis ( $p=0.001$ , permuted  $p=0.025$ ). Homozygous carrier patients of the haplotype had an elevated risk for severe sepsis (odds ratio: 2.21; 95% confidence interval: 1.39-3.51;  $p=0.0006$ ) and this result was unaffected by covariate and population stratification adjustments. These patients also showed a consistent elevation of mean serum LBP concentrations across days examined. Taken together, these results support that common variation in *LBP* gene contributes to susceptibility to severe sepsis. Supported by grants from FUNCIS (37/02) and Ministerio de Educación y Ciencia, Spain (SAF 2004-06833)

**P06.148****Effects of LCAT polymorphisms on HDL-C levels in Turkish population.**

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High density lipoprotein cholesterol (HDL-C) protects from vascular diseases. Genetic and environmental factors influence HDL-C levels. Low HDL-C is common among Turks. Nearly 50 % of the population has HDL-C levels below 35 mg/dl. Lecithin cholesterol acyltransferase (LCAT) takes part in reverse cholesterol transport and HDL-C maturation. The objective of our study is to evaluate the effects of 3 polymorphisms in LCAT gene on HDL-C levels. Study consists of 50 subjects with low HDL-C levels (<35 mg/dl) and 50 control subjects with high HDL-C levels (>65 mg/dl). We studied 511C/T polymorphism (exon 4), 4886C/T (exon 6), 608C/T (exon 5). Although single locus effects of the 3 polymorphisms of LCAT gene were not significantly related to the HDL levels, when combined effects are studied, the subjects with total of less than 2 high risk alleles had significantly higher HDL-C levels compared to subjects with 2 or more high risk alleles ( $56\pm27$  vs  $44\pm23$ ,  $p=0.04$ ). Multiple linear regression was performed to find the predictors of HDL-C. The variables were body mass index (BMI), glucose, age, smoking (number of pack years), number of high risk alleles. Age and smoking were the strongest predictors of HDL-C. However, after controlling for age and smoking, there was a negative correlation between the number of high risk alleles and HDL-C levels with borderline statistical significance ( $p=0.07$ ).

These 3 genetic polymorphisms are susceptibility loci and are neither necessary nor sufficient for prediction of HDL-C, but co-existence of high risk alleles may contribute to low HDL-C levels.

**P06.149****A study of LRRK2 mutations in Parkinson's disease patients from the Volga-Ural region of Russia**

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Mutations in the leucine-rich repeat kinase 2 (LRRK2) gene have been shown to cause both autosomal dominant and sporadic Parkinson's disease (PD). The common G2019S mutation shows wide geographical distribution while G2385R and I2020T variants are suggested to be risk factors for sporadic PD in Asian populations. The frequency of these mutations in the Volga-Ural region of Russia remained to be established. To determine the prevalence of G2019S, G2385R, I2020T mutations in the Volga-Ural region, we recruited 341PD patients (129 Russians, 156 Tatars and 56 Bashkirs) and 360 controls (103 Russians, 131 Tatars, 126 Bashkirs) and conducted genetic analysis of these mutations. All patients exhibited at least two of the four principal signs of PD: resting tremor, rigidity, bradykinesia, or postural-reflex impairment. Exclusion criteria included prior history of multiple cerebrovascular events or other causes of parkinsonian symptoms. Controls were matched for age, ethnic origin and area of residence. Heterozygous G2019S was detected in one late-onset Bashkir patient with akinetic-rigid-trembling form (1.79%). Interestingly, that this patient doesn't carry a SNP in intron 13 (IVS13+104 G/A) which is in strong LD with the mutation and tags the commonest haplotype shared by all patients with this mutation in the Mediterranean populations. The "Asian" risk factor, G2385R, was rare, but found in 2 Tatar PD patients (1.28%). We observed no I2020T mutations in this study sample. All mutations were confirmed by direct sequencing. These data have implications both for understanding the molecular mechanisms of PD, and for directing the genetic screening in clinical practice.

**P06.150****Genetic Diagnosis and investigations of Disease LHON and CPEO**

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Leber hereditary optic neuropathy (LHON) is a maternally inherited form of retinal ganglion cell degeneration leading to optic atrophy in young adults. To investigate any possible association between LHON primary mutations and

mtDNA haplogroups, the nucleotide sequence of the HVS-I region of mtDNA was determined in 30 unrelated Iranian patients with LHON harboring one of the primary mutations and 100 normal controls with the same ethnicity. Our analysis revealed a relatively high proportion of haplogroup J in LHON patients (53.3%) compared to normal controls (20%). In addition, a slightly significant increase of normal controls of haplogroup L has been confirmed (14% in normal controls vs. 0% in LHON patients at  $p < 0.03$ ), whereas other haplogroups did not show contribution to LHON contingency. We identified twelve nucleotide substitutions. Eleven of twelve nucleotide substitutions had already been reported as polymorphism. One of the nucleotide substitutions (A14290G) has not been reported. The A14290G nucleotide substitution does not change its amino acid. Different sizes of mtDNA deletions were detected in 16 patients (69.6%). Each of the 5.5, 7, 7.5 and 9 kb deletions existed only in 1 patient. Common deletion (4977bp) and 8 kb deletion were detected in 5 and 3 patients respectively. Multiple deletions were also present in 4 patients. Out of 23 patients included in our study, two cases (8.7%) had Twinkle gene mutation (G1423C) and 5 patients (21.7%) did not show any deletions in mtDNA or the Twinkle gene mutation.

**P06.151****A visual aura locus for migraine on chromosome 9q31**

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Migraine is the most common cause of chronic episodic severe headache affecting approximately 15 % of the adult population. It has a

well-recognized genetic component based on family and twin studies. However, no genetic variants have been convincingly established for common forms of migraine. In about 30% of all migraine attacks visual, sensory or dysphasic symptoms precede the headache phase manifesting migraine with aura (MA). Here, we present the results of a genome-wide linkage scan on 37 Finnish migraine families having visual aura symptoms. The families were extracted from a large Finnish migraine patient collection ascertained from neurology clinics nationwide during the last 15 years. For this study the families presenting with migraine with typical aura (according to the International Headache Society (IHS) criteria) were selected. Data on IHS defined attack symptoms as well as other clinical features were collected using the validated Finnish Migraine Specific Questionnaire for Family Studies and by a neurologist's examination of index patients. We genotyped 372 individuals using microsatellite markers with an intermarker distance of 10 cM. We detected a locus on 9q31 with significant evidence of linkage (HLOD 4.5 at 104 cM) using two-point parametric linkage analysis. Multipoint parametric and non-parametric analyses support this finding. An additional set of microsatellite markers is currently genotyped to maximize linkage information across the region. In conclusion, our finding indicates a locus for visual aura on 9q31 region previously linked to epilepsy in a Belgian family also suffering from MA.

#### P06.152

#### Haplotype analysis for 12 loci in 11 small Portuguese families with autosomal recessive non-syndromic hearing loss

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Mutations in DFNB1 were shown to account for ~19% of the cases of congenital deafness in the Portuguese population. The cause of deafness in the remaining cases, including the GJB2/GJB6 monoallelic ones, has to be elucidated. We have thus performed linkage analysis in order to investigate the spectrum of genes/mutations responsible for hearing impairment in the Portuguese population.

This study was conducted in eleven small families with apparent non-syndromic congenital deafness. Haplotype analysis was performed for DFNB1, DFNB4, DFNB7/11, DFNB8/10, DFNB9, DFNB12, DFNB18, DFNB28, DFNB35, DFNB49, DFNB59 and DFNB67, using at least three microsatellite markers for each locus. Sequencing analysis of the related genes was carried out in the case of compatibility with linkage.

In a consanguineous family we found compatibility with linkage to DFNB4 with autozygosity for this region. Sequencing of the SLC26A4 gene revealed a novel mutation, c.1615 -2 A>G (IVS14 -2 A>G), in homozygosity in the severely deaf siblings. In a non-consanguineous family, we found compatibility with linkage to DFNB8/10 in heterozygosity. Sequencing of the TMPRSS3 gene revealed a known mutation, c.646C>T (p.Arg216Cys), in compound heterozygosity with a novel mutation that affects the LDLA domain of the protease, c.346G>A (p.Val116Met), in the profoundly deaf siblings. In both families, the genotype is likely to be the cause of deafness.

These are the first DFNB4- and DFNB8/10-related cases of deafness described in Portuguese families, which also provide a first evidence of the genetic heterogeneity of deafness in the Portuguese population.

#### P06.153

#### Combined linkage analyses of four isolated populations suggest novel loci for plasma lipid concentrations

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Despite considerable progress in elucidating genetic influences underlying circulating lipid levels, large proportions of the variance of these traits remain unexplained. In this study, data from four genetically-isolated populations, located in the Netherlands ( $n_{eff}=1218$ ), Italy ( $n_{eff}=918$ ), Sweden ( $n_{eff}=409$ ), and Croatia ( $n_{eff}=537$ ) were synthesized in an effort to increase statistical power to detect genomic regions

which may be involved in total cholesterol (TC), low-, and high-density lipoprotein cholesterol (LDL-c and HDL-c) levels. Quantitative multipoint linkage analyses of the four populations were performed individually, utilizing an integrated map, with SOLAR. LOD scores were combined post-hoc, and adjusted thresholds were calculated to account for multiple testing. Diabetics and subjects receiving lipid-lowering therapy were excluded from analysis. Two models were implemented: age- and sex-adjusted; and age-, sex-, BMI-, alcohol consumption-, and smoking-adjusted. Numerous regions surpassed a suggestive linkage threshold corresponding to LOD=1.9 in either the combined sample or in an individual population. For TC, regions centered on 1q21.3 ( $LOD_{max}=3.21$ ) and 9q31.1 ( $LOD_{max}=2.54$ ) were determined; the chromosome 9 region was also suggestive for LDL ( $LOD_{max}=3.22$ ). HDL peaks exceeded the threshold at 2p21 ( $LOD_{max}=3.77$ ), 10q21.1 ( $LOD_{max}=2.50$ ), 16q12.2 ( $LOD_{max}=3.54$ ), and 21q21.1 ( $LOD_{max}=3.66$ ). The HDL peak on chromosome 2 was also detected for TC/HDL-c ratio ( $LOD_{max}=2.48$ ), as was a peak located at 1p31.2 ( $LOD_{max}=3.74$ ). The latter was also identified for LDL-c/HDL-c ratio ( $LOD_{max}=3.70$ ), in addition to a peak at 22q11.2 ( $LOD_{max}=3.03$ ). These peaks, many of which are novel, contain numerous promising candidate genes. The EUROSPLAN Consortium is currently analysing dense sets of single nucleotide polymorphisms underlying these peaks.

#### P06.154

#### Linkage based on a single family--family size requirement

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Determination of required family size for linkage studies is not a simple task. With genotyping of high-density single nucleotide polymorphism (SNP) markers replacing that of microsatellite markers in linkage studies, it is possible to accurately determine the genomic regions shared by family members, including remote relatives. This may allow linkage studies to be conducted in a more deterministic fashion. Based on this advancement, we developed a simple method to allow the users to evaluate the inheritance information that can be extracted from their diseased family collection in linkage studies. This includes the total number of linked regions expected to be identified for the family under study, the expected sizes for the linked regions, and the portion of the genome that can be excluded from consideration. We presented a detailed discussion on family size requirement for linkage studies by analyzing some typical family structures, and demonstrated the feasibility of linkage studies on smaller families which are thought not sufficient by classical linkage analysis methods. Simulation results by our program showed that the linked regions containing true mutations are usually larger than regions linked due to random chance. We have made use of this feature in our program to allow evaluation of the linked regions through a Bayesian probability calculation.

#### P06.155

#### Genetic diversity and structure of linkage disequilibrium in the MTHFR locus

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Methylenetetrahydrofolate reductase (MTHFR) is a key ferment of folate cycle, which catalyzes for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The reduction to activities of this enzyme, often conditioned presence of the certain combinations of alleles of MTHFR gene, brings to increase the concentrations of homocysteine in blood. In the presented work we explored genetic differentiation and structure of linkage disequilibrium in MTHFR gene in population of the Russians, Tuvinians, north and south Kirghizes, using the set of SNPs: rs17037397, rs4846052, rs1801133, rs1801131 and rs1537516. In the total sample 19 out of 32 possible haplotypes were found (from 10 + 16 per population). The majority of genetic diversity is comprised by 8 main haplotypes, which accounted for 78% of all chromosomes. The population-specific patterns of linkage disequilibrium (LD) at MTHFR locus was revealed. In populations of the Russians, Tuvinians and north Kirghizes 2 LD blocks were found, where as south Kirghizes demonstrates strong linkage of all SNPs, within the single block. The significant genetic differences are discovered between population south Kirghizes and all explored group, as well as between Russians and Tuvinians. Genetic and demographic mechanisms of LD formation in local populations are discussed.

**P06.156****Increasing power of variance component linkage analysis of large pedigrees****T. I. Axenovich, I. V. Zorkoltseva, G. R. Svischeva;***Institute of Cytology and Genetics, Novosibirsk, Russian Federation.*

Most methods of linkage analysis of quantitative traits are based on the variance component approach. IBD calculation is the most computationally intensive step of this approach. The size of IBD matrix for large pedigrees and especially for those coming from isolated populations is too large for practical calculation. To solve this problem a pedigree is split on smaller non-overlapping fragments and then the fragments are analyzed as independent pedigrees. The split leads to increase the number of IBD matrix elements with zero values. This in turn decreases the linkage power. To diminish this effect we suggested to increase the number of non zero elements of IBD matrix by additional alternative splitting of the pedigree, for example, on overlapping fragments. Using the pedigree with 207 members in 6 generations, we estimated the expected linkage power when IBDs were calculated for a) pairs of genotyped members of each non-overlapping fragments of size no more than 18 bit and b) for (a) plus additional pairs of genotyped relatives from overlapping fragments of size no more than 18 bit. The results demonstrated the effectiveness of our method: the power for variant (b) based on 964 IBDs was 1.2 - 1.7 times greater than for variant (a) based on 844 IBDs. Such once calculated IBD matrix can further be utilized by various programs (for example, SOLAR) for linkage analysis of different traits.

**P06.157****A genome-wide association analysis of HDL-cholesterol in the population-based KORA study sheds new light on intergenic regions****I. M. Heid<sup>1,2</sup>, E. Boes<sup>3</sup>, M. Mueller<sup>1,2</sup>, B. Kollerits<sup>3</sup>, C. Lamina<sup>1</sup>, S. Coassini<sup>3</sup>, C. Gieger<sup>1,2</sup>, A. Doering<sup>1</sup>, N. Klopp<sup>1</sup>, R. Frikke-Schmidt<sup>4</sup>, A. Tybjærg-Hansen<sup>4</sup>, A. Brandstätter<sup>3</sup>, T. Meitinger<sup>1</sup>, H. Wichmann<sup>1,2</sup>, F. Kronenberg<sup>3</sup>,**<sup>1</sup>Helmholtz Zentrum München, Neuherberg, Germany, <sup>2</sup>Ludwig-Maximilians-University of Munich, Munich, Germany, <sup>3</sup>Innsbruck Medical University, Innsbruck, Austria, <sup>4</sup>Copenhagen University Hospital, Copenhagen, Denmark.

**Objective:** High-density lipoprotein cholesterol (HDLC) is a strong risk factor for atherosclerosis and assumed to be under considerable genetic control. We aimed to identify gene regions influencing HDLC levels by a genome-wide association (GWA) analysis in the population-based KORA Study.

**Methods:** In KORA S3/F3 (n=1,643), we analyzed the 377,865 quality-checked single nucleotide polymorphisms (SNPs) of the 500K Affymetrix SNP array, complemented by the publicly available GWA results from the Diabetes Genetics Initiative (DGI, n=2,631) and by replication studies in KORA S4 (n=4,037) and the Copenhagen City Heart Study (n=9,205).

**Results:** Among the 13 SNPs selected from the KORA S3/F3 500K p-value list, three SNPs showed consistent associations in subsequent replications: two from so far unreported regions 50 kb downstream of LIPG or upstream of CETP and one from already reported regions of the LPL. The SNP in CETP and several SNPs downstream of LIPG were independent from already reported SNPs and might be of functional relevance. The other 10 as well as further 13 SNPs selected from the combined GWA p-value list of KORA S3/F3 and DGI failed consistent replication.

**Conclusions:** Our GWA study identified two new HDLC-relevant regions, which generally motivates to extend the focus of future genetic association studies on long-range effects of intergenic regions. Furthermore, our study reinforced CETP and LPL as strong HDLC genes and thereby underscores the power of our study and of the GWA approach in general to pinpoint strong effects from common polymorphisms.

**P06.158****Genetic variants in the USF1 gene are not associated with MetS, T2DM, and related parameters in Caucasians (KORA study)****N. Klopp<sup>1</sup>, C. Holzapfel<sup>1</sup>, H. Grallert<sup>1</sup>, C. Huth<sup>1</sup>, C. Gieger<sup>1</sup>, C. Meisinger<sup>1</sup>, K. Strassburger<sup>2</sup>, G. Gian<sup>2</sup>, H. Wichmann<sup>1</sup>, C. Herder<sup>2</sup>, W. Rathmann<sup>2</sup>, T. Illig<sup>1</sup>,**<sup>1</sup>Helmholtz Zentrum München, Munich-Neuherberg, Germany, <sup>2</sup>German Diabe-

tes Center, Leibniz Institute at Heinrich-Heine-University, Düsseldorf, Germany. Upstream stimulatory factor 1 (USF1) regulates numerous genes of glucose and lipid metabolism and genetic variants in the USF1 gene show association with familial combined hyperlipidemia, which shows phenotypic overlap with the metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM). The aim of our study was to approve the hypothesis that polymorphisms in the USF1 gene are associated with MetS and related metabolic traits. We genotyped eight single nucleotide polymorphisms in the USF1 gene in 1,653 individuals of the population-based German Caucasian KORA study in the age range between 55 and 74 years. Because of high correlation only six polymorphisms were statistically analyzed. The association with T2DM and the MetS was analyzed by logistic regression in 1,462 subjects and the quantitative parameters were analyzed in 1,231 fasting non diabetic subjects by linear regression respectively by Kruskal-Wallis test. None of the analyzed genetic variants (rs2774279, rs10908821, rs1556259, rs2516839, rs3737787, rs2774276) show significant association with T2DM and MetS. The results for the metabolic traits like triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, percent body fat, waist to hip ratio, ureic acid, fasting glucose, 2 hour plasma glucose, fasting insulin, blood pressure give also no evidence for any association with USF1. In our large population based study no association between single genetic variants in the USF1 gene and T2DM, MetS and related metabolic parameters was found. We conclude that the single genetic variants in USF1 have no major effect on lipid and glucose parameters in German Caucasians.

**P06.159****Genome-wide linkage analysis for identifying quantitative trait loci involved in the regulation of lipoprotein a (Lpa) levels****S. Lopez<sup>1</sup>, A. Buil<sup>1,2</sup>, J. Ordoñez<sup>3,4</sup>, J. C. Souto<sup>1</sup>, L. Almasy<sup>5</sup>, M. Lathrop<sup>6</sup>, J. Blangero<sup>5</sup>, F. Blanco-Vaca<sup>3,7</sup>, J. Fontcuberta<sup>1</sup>, J. M. Soria<sup>1,2</sup>,**<sup>1</sup>Haemostasis and Thrombosis Unit, Department of Hematology, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>2</sup>Bioinformatic of Complex Diseases Unit, Research Institute of Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>3</sup>Department of Biochemistry, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, <sup>4</sup>Department of Biochemistry and Molecular Biology, Universitat Autònoma de Barcelona, Barcelona, Spain, <sup>5</sup>Department of Population Genetics, Southwest Foundation for Biomedical Research, San Antonio, TX, United States, <sup>6</sup>Centre National du Génotypage, Paris, France, <sup>7</sup>CIBER de Diabetes y Enfermedades Metabólicas Asociadas, Barcelona, Spain.

Lipoprotein Lp(a) levels are highly heritable and are associated with cardiovascular risk. We performed a genome-wide linkage analysis to delineate the genomic regions that influence the concentration of Lp(a) in families from the Genetic Analysis of Idiopathic Thrombophilia (GAIT) Project. Lp(a) levels were measured in 387 individuals belonging to 21 extended Spanish families. A total of 485 DNA microsatellite markers were genotyped to provide a 7.1 cM genetic map. A variance component linkage method was used to evaluate linkage and to detect quantitative trait loci (QTLs). The main QTL that showed strong evidence of linkage with Lp(a) levels was located at the structural gene for apo(a) on Chromosome 6 (LOD score=13.8). Interestingly, another QTL influencing Lp(a) concentration was located on Chromosome 2 with a LOD score of 2.01. This region contains several candidate genes. One of them is the *tissue factor pathway inhibitor (TFPI)*, which has antithrombotic action and also has the ability to bind lipoproteins. However, quantitative trait association analyses performed with 12 SNPs in *TFPI* gene revealed no association with Lp(a) levels. Our study confirms previous results on the genetic basis of Lp(a) levels. In addition, we report a new QTL on Chromosome 2 involved in the quantitative variation of Lp(a). These data should serve as the basis for further detection of candidate genes and to elucidate the relationship between the concentration of Lp(a) and cardiovascular risk.

**P06.160****Identification of a novel autosomal recessive locus on chromosome 5q31-34 for the long QT syndrome****Z. N. Al-Hassnan<sup>1</sup>, S. Majid<sup>1</sup>, M. Al-Fayyadh<sup>2</sup>, N. Dzimiri<sup>1</sup>, Y. Mallawi<sup>1</sup>, W. Al Mana<sup>1</sup>, M. Al-Owain<sup>1</sup>, B. Meyer<sup>3</sup>,**<sup>1</sup>King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

It is estimated that a significant percentage of sudden deaths are due to the long QT syndrome (LQTS) which is an inherited arrhythmic disorder that is characterized by locus heterogeneity. Mutations in

several genes that code for ion channels have been implicated in the LQTS which exists in two main forms: (i) Romano Ward syndrome inherited as autosomal dominant, and (ii) Jervell and Lange-Nielsen syndrome, an autosomal recessive disorder which is associated with sensorineural deafness.

Here we present result of genetic linkage analysis on a family with a history of sudden death in three children at the age of 7 to 8 years. Screening the other four living children and their consanguineous parents revealed that two sons were affected with prolonged QT intervals with unremarkable hearing assessment. Whole genome scan using Allegro software identified a locus on chromosome 5q31-34 with a maximum positive LOD score of 2.05. This locus has not been described in association with the LQTS. Further analysis of the region with sequencing of potential candidate genes is in progress.

In this work, we report on the identification of a novel locus for the LQTS. In the absence of hearing loss, the autosomal recessive pattern of inheritance in our family suggests a new form of the LQTS. Furthermore, identifying the causative gene on this locus may provide insight into the genetic susceptibility to unexplained sudden death in general.

#### P06.161 LRP1, a candidate gene for risk modulation of premature cardiovascular disease in heterozygous familial hypercholesterolemia

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Heterozygous familial hypercholesterolemia (hFH) is characterized by severely elevated LDL-cholesterol levels and premature atherosclerosis and cardiovascular disease (PCVD). Genetic alterations of the LDL-receptor gene are the main cause of this disease. However, there is a substantial variation in the onset and severity of the disease. This variability might be due to atherogenic risk factors of environmental, metabolic and genetic origin modulating the phenotype of hFH disease. LDL-receptor-related receptor (*LRP1*) is a multiligand binding receptor that acts as a pivotal lipoprotein receptor for the atherotrombotic transformation of the vascular wall. The aim of this study was to analyse the association of *LRP1* polymorphisms with premature cardiovascular disease in 339 hFH-patients (262 PCVD and 77 non-PCVD) from the Spanish FH Register. The polymorphisms c.1-25 C>G, rs715948, rs1799986, rs1800127, rs7968719, rs1800176, rs1800194, rs1800181, rs1140648, rs1800164 distributed along the whole gene were used in the study. The polymorphism rs1799986 showed a significant association with PCVD in the codominant model for the heterozygous genotype CT (nominal  $P = 0.0126$ , OR 2.07. CI<sub>95</sub> 1.14-3.74). The functional effect of rs1799986, that apparently is a synonymous mutation (Asp100Asp), was analyzed by the web interface <http://pu-pasuite.bioinfo.cipf.es>. This polymorphism completely abolishes an Exonic Splicing Enhancer sequence. This may results in splicing aberrations as exon skipping and therefore in a deficient transcription of the gene. Although further studies are needed to confirm the rs1799986 association with atherosclerosis and cardiovascular risk and to clarify its physiological role, our results suggest that this polymorphism is a biological plausible risk factor for PCVD.

#### P06.162 No association between polymorphic loci of the G72 gene and major depressive disorder

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Major depressive disorder (MDD) is a common and highly heterogeneous psychiatric disorder encompassing a spectrum of symptoms involving deficits to a range of cognitive, psychomotor and emotional processes. The flavoprotein D-amino acid oxidase (DAO) degrades the gliotransmitter D-Ser, a potent activator of N-methyl-D-aspartate-type glutamate receptors. A body of evidence suggests that DAO, together with its activator, G72 protein, may play a key role in the pathophysiology of MDD. The present study tested the hypothesis that the M23 and M15 polymorphic loci of the G72 gene are associated with major depressive disorder (MDD). We studied M23 and M15 polymorphic loci of the G72 gene in 163 MDD patients and in 268 normal controls

from Bashkortostan region (belonged to Russian and Tatars ethnic groups). No significant differences were demonstrated for genotype or allele frequency of the M23 and of M15 polymorphic loci of G72 gene comparing the MDD and control group. The patient and the control samples were not divided according ethnicity, because genotype and allele frequencies were coincide in patient and control sample. Haplotype analysis was conducted to assess the association between the two markers within the G72 gene. The results showed that these two markers are in no linkage disequilibrium (LD): D' (patient sample) = 0.056, D' (control sample) = 0.064. Our findings suggest that M23 and M15 polymorphic loci of the G72 gene do not play a major role in the susceptibility to MDD. However, further studies with a larger sample are required to confirm the obtained results.

#### P06.163

#### Search for genetic mechanisms involved in malaria infection in Amazon

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The biochemical pathways involved in the pathogenesis of the parasite infection that causes malaria and the human host mechanisms of defense against it are still not entirely known. Epidemiologic studies, as well genetic studies, suggest the existence of genetic components related to the host innate resistance or susceptibility to malaria (Hill A.V., 2006). Based on results obtained by our group in a genome-wide scan in 182 individuals from Portuchuelo (8°37'S, 63°49'W), Rondonia state, Brazil (Ferreira R.G.M. et al., 2007). The following STRs markers in the short arm of chromosome 4 spanning from 0 to 57cM where selected to be genotyped in a distinct population, Monte Negro (10°15'S, 63°18'W), at the same Brazilian state.

The sample, 452 individuals from nuclear families, was genotyped using 7 STRs markers (D4S412 - 4.74cM, D4S2983 - 17.5cM, D4S403 - 25cM, D4S419 - 30cM, D4S3022 - 36cM, D4S391 - 43.6cM, D4S405 - 57cM) along short arm of chromosome 4, with a mean distance of 8.74cM from each other. The software SimWalk2 (Sobel E. et al., 2002) was used to check mendelian segregation of the alleles in the families.

The reported number of malarial episodes, corrected by age and sex, was used as the quantitative trait phenotype. This phenotype showed expected association with Fy- individuals as well as familial aggregation in conformity with genetic mechanisms, indicating its epidemiological importance. Linkage analysis were conducted using the software Solar 2 (Almasy L. et al.; 1998).

No evidence of linkage was found both in multi-point and two-point analysis. (FAPESP,CNPq)

#### P06.164

#### Matrix Metalloproteinase-3 gene polymorphism and dilatative pathology of ascending thoracic aorta

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The aim of the study was to identify whether the mutation in the promoter region of MMP3 gene might be a determinant of dilatative pathology of ascending thoracic aorta (DP ATA).

Material and methods. We studied 76 (55 males, 21 females) patients with DP ATA, the age ranged from 31 to 81 years (median, 64 years) and a random sample of the population ((244 males and 366 females) aged 25-64 yrs., all from Lithuania. Analysis was done on DNA using real-time polymerase chain reaction to genotype polymorphism 5A/6A at a position -1171 of the MMP3 gene promoter.

Results. The prevalence of MMP-3 genotypes was similar between group of DP ATA and random sample of population. The frequency of 5A allele did not differ between both groups and was 0.506 and 0.514, respectively. Male patients carrying 5A/5A genotype were significantly younger compared with those with the 6A/6A genotype.

In conclusion, the frequency of MMP-3 promoter 5A/6A genotypes did not differ between the group of patients with DP ATA and the random sample of population, but the males with DP ATA and 5A/5A genotype required aortic reconstruction surgery at the younger age than the

males carrying 6A/6A genotype in the MMP-3 promoter region.

#### P06.165

#### Matrix metalloproteinase-9 polymorphism in relation to cardiovascular disease complications in patients of type 2 diabetes mellitus

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The process of atherosclerosis is accelerated in patients of type 2 Diabetes Mellitus (T2DM). The traditional risk factors alone cannot explain the excess incidence of cardiovascular complications in T2DM patients. The matrix metalloproteinase 9 (MMP-9) gene promoter genotype was considered to be related to coronary artery disease. Our study aimed to evaluate possible role of C-1562T MMP-9 polymorphism in developing of cardiovascular complications among T2DM patients. We have studied 111 subjects with C/C (group 1) and 50 subjects with T/T (in 1 patient) or C/T genotype (group 2). Fasting serum glucose and lipid metabolism parameters, inflammatory markers and anthropometric data were measured. MMP-9 genotype was detected by the PCR method. Cardiovascular complications were proven angiographically. Results. A cardiovascular complication was detected in 45% among C/C homozygotes and 48% among patients of group 2 (C/T+T/T). Waist/hip ratio and diastolic blood pressure were significantly higher in T allele carriers ( $p<0.05$ ). In multiple regression analysis independent determinants of diastolic blood pressure were MMP-9 genotype, waist/hip ratio and triglycerides level.

Conclusion. Among diabetic patients carrying MMP-9 T allele were revealed some unfavourable changes in anthropometric, lipid and blood pressure data, which can play a certain role in genesis of cardiovascular complications.

#### P06.166

#### Analysis of assembly of A1903G of gene polymorphism of chymase of CMA1/B with maximal oxygen consumption in human subject

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Introduction: the chymase gene of CMA1/B locates on 14 chromosomes (14q11.2) and the chymase is the main enzyme, controlling the angiotensin II generation from angiotinin I in tissues of cardiovascular system. The analysis of assembly of A1903G of gene polymorphism of chymase of CMA1/B with people physical characters has been carried out.

Methods: maximal oxygen consumption (MOC) of the 100 high-skilled sportsman's in the age 17-25 year had been investigated using Guminsogo method. The group of comparison consisted of 100 healthy individuals of appropriate age, which are not interested in sport (control group). The analysis of genetic polymorphism realized by the polymerase chain reaction (PCR).

Results: the genotypes 1)\*A/\*A, 2)\*A/\*G, 3)\*G/\*G chymase gene of CMA1/B are the most frequent 1)26,25%, 2)60%, 3)13,75% in the group of sportsman's; 1) 50%, 2)50%, 3)0% in control group with low means of MOC. We revealed that the reliable frequency the allele \*G rise till mean (43.75% against 25% in control group was found; P=0.506; OR=1.751, 95%CI 1.102-5.0) and the allele \*A low till mean (56.25% against 75% in control group; P=0.0506; OR=0.751, 95%CI 0.20-0.907). Also revealed the genotype \*A/\*A low till mean 26.25% against 50.0% in control group; P=0.0886; OR=0.525, 95%CI 0.129-0.977).

Is known, that the presence in allele genotype of CMA\*G leads to the decrease of education of active pressor peptide - angiotensin II. Conclusions: the genotype association of CMA\*G/\*G of chymase gene with high level of MOC was determined, that provides of full value supply of heart tissue by the oxygen on exertions.

#### P06.167

#### Is Mannose Binding Lectin Codon 54 Polymorphism

#### Associated With Predisposition to Acute Poststreptococcal Glomerulonephritis in Childhood?

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Acute Poststreptococcal Glomerulonephritis (PSAGN) is the most common form of glomerulonephritis, a disorder marked by inflammation of the kidneys' glomeruli, following a streptococcal infection. However the mechanism of renal injury in PSAGN is still not clear. On the other hand Mannose binding lectin (MBL) is a calcium dependent lectin that has an important role in innate immunity. MBL deficiency increases the overall susceptibility of an individual to infectious disease. The aim of this study is to determine the presence of any association between MBL gene variants and PSGAN in the child population. Codon 54 (allele B) polymorphism in the exon 1 of the MBL gene was investigated by PCR-RFLP method in 110 children diagnosed as PSGAN and 100 age-matched healthy controls. No significant differences in the frequencies of the mutant B allele were found between the patient group (5.5%) and the control group (1%). AB genotypes was found to be 20% and 26% in the patient group and the healthy control group respectively, where the difference was statistically not significant. AA genotype was found in 74.5% of the children with PSGAN and 73% of the healthy control group. In our study, we could not find any significant association between MBL genotypes and PSGAN. We conclude that it may be helpful to test the association between MBL genotypes and other infectious diseases in a large series of patients in order to resolve the role of MBL protein in susceptibility to or protection against infections with different pathogens in certain age groups

#### P06.168

#### Medullary Cystic Kidney Disease 1 - The quest for finding the gene

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Medullary Cystic Kidney Disease (MCKD) is an autosomal dominant disorder manifesting with interstitial fibrosis, tubular basement membrane thickening and tubular atrophy, leading to CRF or progressing to ESRF at ages between 30-70 years. Renal cysts at the corticomedullary junction are a frequent finding. In certain regions of Pafos, Cyprus, MCKD1 patients constitute nearly 40% of total ESRF cases. The gene responsible for the disease, MCKD1, has been mapped by our group on 1q21 (Christodoulou et al, 1998, Hum Mol Genet 7:905-911), between markers D1S498 and D1S2125. Paucity of progress in cloning the gene, prompted us to re-evaluate and expand linkage analysis data encompassing patients from nine families linked to MCKD1. All patients were examined using global criteria in an attempt to identify an intermediate phenotype. Polymorphic markers were examined throughout the MCKD1 region and haplotypes helped to re-establish the critical region, between markers D1S305-D1S336mw28, encompassing 2.1 Mb. Genes considered as good candidates, NP12, PIAS3, IL6R, APOA1BP, CA14, NCu-GI, FDPS, HCN3, AQP10, SHC1, PMVK, SCAMP3, LOC284620 and MUC1, were re-sequenced, with negative results. Additionally, a microarray expression study using renal fibroblasts from an affected kidney was designed in order to depict panoramic gene expression, also with no considerable results. A Comparative Genome Hybridization array experiment was performed searching for allelic copy number variations nested in the critical MCKD1 region. No significant difference was detected between normal and affected samples. From the clinical perspective, this work revealed a putative relationship between carriers of affected haplotype and hyperuricemia, as an early clinical finding.

**P06.169****Absence of causative coding variants in the MEIS1 gene in 245 French-Canadian Restless Legs Syndrome patients**

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**Introduction:** Restless legs syndrome (RLS) is a common sensorimotor disorder with a substantial genetic contribution. Recently, a genome-wide association study reported significant association between common variants within the MEIS1 gene and the RLS phenotype. The odds ratio of the risk allele ranges from 1.8 to 3.0. The MEIS1 gene encodes a homeobox protein involved in limb development, establishment of the intrasegmental spinal motor neuron identity and connectivity, and patterning of sensory organs in the embryonic peripheral nervous system, as well as hematopoiesis.

**Patients and methods:** We systematically sequenced 245 clinically well-defined RLS patients for the entire coding and UTR regions of the MEIS1 gene. All samples were Caucasians with 95% of French-Canadian (FC) origin; and 77% were severe or very severe cases with mean age of 53±12 yrs and disease onset 30±16 yrs. 77% of patients had positive family history and 83% were also positive for periodic leg movements during sleep. In total, 13 exons/17 fragments (NM\_002398.2, 3180 bps) were sequenced. All sequence variants detected in patients were genotyped in 285 FC controls.

**Results:** In total, 19 variants were detected, five intronic and 14 exonic, including 13 variants within the 3'UTR region and one synonymous SNP (rs13005707) in exon 7. Eight rare variants and eleven common variants showed no association with the disease phenotype.

**Conclusion:** No causative coding variants were found in the MEIS1 gene in 245 RLS patients. Further deep-sequencing of the non-coding region and studies of the expression and function of the MEIS1 gene are underway.

**P06.170****Effect of polymorphisms in the dopaminergic system genes on the mental fatigue**

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Mental fatigue (MF) represents a failure to initiate and sustain tasks that require self-motivation and internal cues in the absence of demonstrable cognitive failure or motor weakness. So development of the mental fatigue is the significant problem for people who work with big amount of data and constantly need focused attention.

Molecular mechanisms of MF origin still are not clear. It is known that basal ganglia play a key role in the development of MF (Chaudhuri et al., 2000). Dopamine is one of the most prevalent neurotransmitters in the basal ganglia. Therefore we suppose that alterations in the functioning of dopamine system provided by genes variations may influence the development of the MF. We tested the hypothesis that allelic variation of the DRD2 and DAT genes located mostly in the striatum could be associated with differences in the onset of MF.

In current investigation 160 volunteers (78 males and 82 females) participated. Subjects were genotyped for TaqI A RFLP of the DRD2 gene and 40-bp VNTR polymorphism of the DAT gene. Psychophysiological indexes were measured before and after mental load consisted in the monotonous passing of personality questionnaires during three hours.

Case-control analyses suggested a strong association between the A1+10/10 genotype and increased mental fatigue ( $P < 0.05$ ). In conclusion, mental fatigue seems to be related to allelic variations within the DRD2 and DAT genes.

**P06.171****Effect of genes from the serotonin system on the mental fatigue**

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Fatigue is an important factor affecting sporting and mental performances. It is complicated process which includes peripheral and central components. Central fatigue occurs in central nervous system as a result of mental as well as physical activity.

There are evidences that serotonin is implicated in central fatigue de-

velopment. Serotonin turnover in the brain increases in response to physical exercise. Decreased motivation, tiredness, loss of motor coordination - markers of central fatigue, are associated with the rise of brain serotonin concentration. But the role of serotonin in the development of mental fatigue is unclear.

In the current study we investigate associations of serotonin system genes polymorphism with the mental fatigue. In the study students of Moscow State University took part (N=160, male=78, female=82). Volunteers were exposed to intensive mental workload (logical tasks and monotonous persistent completion of psychological tests) during 3 hours. Before and after workload the psychophysiological state was estimated with the help of "NS-Psychotest" (Neurosoft, Russia). Additionally volunteers completed self-ratings of mental fatigue and of functional state.

It was detected that carriers of both ss-genotype of the 5HTTLPR and CC-genotype of the 5-HT2A polymorphism (T102C) reported higher level of mental fatigue after performing fatigue task compared with carriers of II(5HTTLPR) genotype and of TT(T102C) genotype.

ss-genotype of the 5-HTTLPR as well as CC-genotype of the 5-HT2A are associated with reduced expression of both genes and therefore could cause increasing concentrations of extracellular serotonin in the brain. These findings suggest a role of serotonin transmission efficiency in mental fatigue.

**P06.172****Joint analysis of individual-level data data from 17 studies on the association of the IL6 promoter polymorphism -174G>C with glucose levels, interleukin-6 levels, and body-mass index**

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**Background:** Several studies have investigated associations between the -174G>C polymorphism (rs1800795) of the IL6 gene and phenotypes related to type 2 diabetes mellitus (T2DM), but presented inconsistent results. This joint analysis aimed to clarify whether IL6 -174G>C was associated with the quantitative phenotypes circulating glucose, body-mass-index (BMI), and circulating interleukin-6 (IL-6).

**Methods:** Individual participants' data from all published and unpublished studies of the IL6-T2DM consortium on Caucasian subjects with available BMI were collected. As study-specific estimates did not show heterogeneity ( $P > 0.1$ ), they were combined by using the inverse-variance fixed-effect model.

**Results:** The main analysis included 9440, 7398, 24,117, or 5659 non-diabetic and manifest T2DM subjects for fasting glucose, 2-h glucose, BMI or circulating IL-6, respectively. The IL6 -174 CC- and GC-genotypes were significantly associated with -0.091mmol/L ( $P=0.014$ ) lower fasting glucose. There was no evidence for association between IL6 -174G>C and BMI or IL-6 levels. In an additional analysis of 641 subjects known to develop T2DM later on, the IL6 -174 CC-genotype was associated with higher baseline IL-6 levels (+0.75pg/mL,  $P=0.004$ ), which was consistent with higher IL-6 levels in the 966 manifest T2DM subjects (+0.50pg/mL,  $P=0.044$ ).

**Conclusions:** Our data on the widely debated IL6 -174G>C polymorphism suggest an association with quantitative glucose, and exploratory analysis indicated modulated IL-6 levels in pre-diabetic subjects,

being in-line with this SNP's previously reported T2DM association and a role of circulating IL-6 as intermediate phenotype. This large joint analysis underscores that a consortium-based approach is well-suited to investigate small genetic effects.

#### P06.173

#### Apolipoprotein A5 gene APOA5\*2 haplotype variant confers risk for the development of metabolic syndrome

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The metabolic syndrome may affect 18-30 per cent of the adult population in the industrialized countries and is considered a veritable epidemic. Metabolic syndrome 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. The etiology is complex, determined by the interplay of both genetic and environmental factors. Naturally occurring variants of the apolipoprotein A5 gene have been associated with increased triglycerides 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, and the haplotype profiles were studied in 343 patients with MS and 284 controls. Minor alleles of all apoA5 variants, except the C56G variant, were associated with increased triglyceride levels both in the MS patients and in the controls. The serum total cholesterol levels did not show allele-dependent differences. The prevalence of the apoA5\*2 haplotype, defined by the simultaneous presence of -1131C, IVS3+473A and 1259C variants, was 13.4% in MS patients and 4.91% in the controls ( $p<0.001$ ). Multiple logistic regression analysis revealed that this haplotype confers risk for the development of MS ( $OR=2.913$ ; 95% CI: 1.564-5.426;  $p<0.001$ ). To our surprise, the apoA5\*5 was significantly less frequent in the MS patients compared with the controls (0.87 vs 5.26%,  $p<0.05$ ), and this haplotype was found to confer strong protection against the development of MS ( $OR=0.158$ ; 95% CI: 0.045-0.553;  $p=0.004$ ).

#### P06.174

#### Polymorphisms of methyl-group pathway genes in spontaneous abortions

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It's believed that altered methyl-group pathway provides a potential mechanism which influence the DNA methylation of specific genomic regions. Thereafter aberrant methylation can lead to the induction of genomic instability in a manner that have developmental consequences including embryo death. The purpose of current work was to investigate the effect of fetal functional polymorphisms of methyl-group pathway genes (C667T and A1298C *MTHFR*, A2756G *MTR*, G66A *MTRR* and C46359T *DNMT3B*) on spontaneous abortions. First-trimester spontaneous abortions with normal karyotype ( $n=151$ ), with mosaic chromosomal constitution ( $n=34$ ) and induced abortions ( $n=33$ ) were analyzed for chosen polymorphisms by PCR-RFLP. Nothing differences between alleles and genotypes frequencies in studied groups were found, except *DNMT3B*. Carriers of 46359T allele predominated in the group of induced abortions (64%) in compare with spontaneous abortions (47%;  $P=0.01$ ). The 46359TT genotype was more prevalent in the group of induced abortions than spontaneous abortions (42% vs. 17%, respectively;  $P=0.002$ ). Consequently, further studies are needed to clarify the influence of embryo genotype for polymorphisms C46359T *DNMT3B* in the process of human spontaneous abortion.

#### P06.175

#### Genetic analysis of autosomal recessive primary microcephaly in Pakistani kindreds

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Autosomal recessive primary microcephaly (MCPH) is a rare genetic disorder in which the affected individuals have head circumference  $>3$  SDs below the age and sex-related mean. The reduced head circumference is due to small but architecturally normal cerebellar cortex. This disorder is genetically heterogeneous with six loci and four genes (Microcephalin, CDK5RAP2, ASPM and CENPJ) have been identified. Its incidence is ranging from 1/30,000 to 1/2,000,000.

Eleven families of autosomal recessive primary microcephaly were ascertained from various areas of Pakistan. By PCR (Polymerase Chain Reaction) based genotyping linkage was performed using highly polymorphic fluorescently labeled microsatellite markers. Homozygosity mapping revealed linkage of seven families to MCPH5 locus (ASPM gene), one to MCPH2 locus, one to MCPH4 locus and one with MCPH6 locus (CENPJ) and one was excluded to all known MCPH loci. MCPH5 linked families were subjected to screen the ASPM (Abnormal Spindle-like Microcephaly Associated) gene with direct DNA sequencing. Four previously reported (p.Y3163X, p.R1019X, p.Y3353X, p.W1326X) and two novel mutations (p.L333fs, p.Q2880X) were identified in ASPM gene. One known mutation (p.T6fsX3) was also identified in CENPJ (Centromere-associated Protein J) gene. All these mutations are protein truncating so it is more likely that they are governing the same phenomenon of nonsense mediated mRNA decay. The ultimate objective of our study is to identify the novel locus in a family which is excluded to all known MCPH loci and to identify the genes responsible for autosomal recessive primary microcephaly in MCPH2 and MCPH4 linked families.

#### P06.176

#### Genetic Variation in miRNA Genomic Regions: Association with Eating Disorders

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MicroRNAs (miRNAs) are posttranscriptional regulators involved in the regulation of at least a third of mammalian genes. Association studies using single nucleotide polymorphisms (SNPs) in miRNA genomic regions might help to evaluate the contribution of functional miRNA allele variants to complex disease susceptibility. We constructed a panel of 768 SNPs covering 164 miRNA regions and spanning 2 Mb of genomic DNA including 326 known human miRNAs precursor sequences (MiRBase, 7.1) and at least 5 kb upstream and downstream of the miRNA regions. For an optimal selection of informative SNPs we used tagSNPs and included 18 SNPs located inside pre-miRNA sequences. Genotyping of 340 unrelated Spanish controls was performed using a custom Golden Gate assay (Illumina). Nine out of the 18 SNPs located in pre-miRNAs were monomorphic, which is suggestive of selective constraint on miRNA sequences. A high correlation ( $R^2=0.86$ ) was shown between allele frequencies in the Spanish and HapMap CEU sample confirming the applicability of our SNP panel to study the Spanish population. Further, we performed association analysis in a sample of 294 patients with different types of eating disorders and 340 controls. A nominal association ( $p<0.005$ ) was found for at least two SNPs per miRNA in the case of two miRNAs and 2 clusters containing 2 and 3 miRNAs each. When stratifying for bulimia and anorexia phenotypes, association for another 3 and one miRNAs was respectively shown. These results indicate a possible role for miRNAs in the aetiology of eating disorders. Supported by Spanish Government and Generalitat de Catalunya.

**P06.177****Sequence analysis of circadian clock modulators miR-132 and miR-219 and their targets RFX4 and PHLPP in Mood Disorder Patients**

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It has been widely supported that circadian rhythms are involved in the pathophysiology of mood disorders (MD). Two brain-specific miRNAs (microRNAs) have been recently reported as modulators of endogenous circadian clock located in the suprachiasmatic nucleus in mice. The authors have functionally characterized miR-132 and miR-219 within the context of circadian clock and gave experimental evidences of genes Rfx4 and Phlpp as respective targets. We first explored the conservation between species for both miRNAs and once the existence of hsa-miR-132 and hsa-miR-219 was corroborated in humans, we used different prediction programs to check the experimentally supported target sites RFX4 and PHLPP in humans. Specifically, miRanda predicted hsa-miR-132 to target RFX4 and TargetScan predicted PHLPP as a target site for hsa-miR-219, consistent with previous work in mice. Once both miRNAs with their respective target sites were confirmed in humans, we questioned whether these two miRNAs and their target genes RFX4 and PHLPP are altered in mood disorder patients. Thus, we re-sequenced both miRNAs and their respective target sites at RFX4 and PHLPP genes in a sample consisting of 365 unrelated patients (218 Unipolar Major Depressive Disorder and 147 Bipolar Disorder) diagnosed according to DSM-IV criteria. All patients completed the Spanish versions of the Seasonal Pattern Assessment Questionnaire and the Horne-Östberg Morningness-Eveningness Questionnaire. This is the first time that the circadian clock regulation by miRNAs is explored in mood disorder, representing a first step to elucidate the underlying mechanism of these proteins and the role of these miRNAs in mood disorders.

**P06.178****Allelic frequencies and heterozygosities of microsatellite markers covering the whole genome in the Korean**

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Microsatellite markers are an essential tool for genetic linkage analysis because of their high polymorphism content. Four hundred commercially available markers covering the entire genome were genotyped from 578 sib individuals from 249 Korean families. Allelic frequencies and heterozygosities were determined for each marker loci and compared between Korean, Taiwanese, Japanese and Caucasian populations. In the three Asian populations, 10-13% of the markers had less than 0.6 heterozygosity, whereas in the Caucasian population, only 0.5% of the markers had less than 0.6 heterozygosity. Mean identical by descent (IBD) values were calculated for 578 sib individuals. Analysis of IBD values greater than 0.5 suggested that markers with low heterozygosity can also provide positive linkages, at least for the IBD sharing method of model-free linkage analysis. The data presented in this study will be a useful reference for genome-wide screens of Koreans and comparative studies with other ethnic populations.

**P06.179****Association study of migraine and 19 candidate genes involved in serotonergic neurotransmission in a Spanish population**

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Migraine is a common neurological disorder with a complex inheritance pattern. Although the pathophysiology of the disease is not well understood, several studies suggest that serotonin-related genes may participate in its pathogenesis. The involvement of some of these genes has been studied in different migraine populations with conflicting results thus far.

In the present study we aimed to evaluate the possible role of 19 genes involved in serotonergic neurotransmission in the susceptibility to migraine in a Spanish population using a case-control approach based on SNPs. A total sample of 528 unrelated patients were recruited and diagnosed following the II<sup>nd</sup> International Criteria of Headache Disorders from the IHS (308 patients had migraine without aura and 220 had migraine with aura) and compared to 528 sex-matched controls in which migraine was ruled out. All individuals were Spanish of Caucasoid origin from Catalonia and Galicia. SNP selection was based on genetic coverage parameters and genotyping was performed using SNplex technology. Chi-square tests were used for the comparison of allele and genotype frequencies between the patient and control groups for each single SNP. Preliminary data of nominal associations suggest a contribution of *HTR1E*, *HTR2B*, *HTR3B*, *MAOA* and *DDC* in the genetic predisposition to migraine.

**P06.180****Genetic diagnosis of Ataxia Telangiectasia and role of Mitochondria on it**

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Ataxia telangiectasia (AT) is an autosomal recessive disorder in 1/40,000 to 1/100,000 in reported populations. There is a 25% possibility for having an affected child when parents are carrier for the ATM gene mutation. There is no cure available for this disease and prenatal testing is strongly recommended for prevention of this disease. 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. In this study, four molecular markers: D11S2179, D11S1787, D11S535, D11S1343 are genotyped in 19 unrelated families from different regions of Iran. All carriers and affected patients were diagnosed accurately. 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 was extracted from whole blood and 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.

**P06.181****Role of mitochondria in Friedreich Ataxia**

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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. Mitochondrial DNA (mtDNA) could be considered a candidate modifier factor for FA disease, since mitochondrial oxidative stress is thought to be involved in the pathogenesis of this disease. The expansion (GAA) repeat in the first intron causes decreased frataxin expression by interfering with transcription.: Activity of mitochondrial respiratory chain complex I (measured as NADH ferricyanide reductase) and intracellular ATP measurement was performed on lymphocyte of FA patients (n =12) and control subjects (n =25). 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. 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.

#### P06.182

##### The developmental changes in mitochondrial DNA content per cell in human liver and muscle tissue during gestation

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The process of oxidative phosphorylation system (OXPHOS) maturation is still not fully understood during the development of the fetus. Since all mammalian cells except erythrocytes depend on mitochondrial ATP production, knowledge of the OXPHOS maturation is an essential query. Our previous study demonstrated significant changes of mitochondrial DNA (mtDNA) content in cord blood leukocytes during the gestation. To further understand the role of mtDNA content in human prenatal development, we analyzed mtDNA amount in human fetal liver and muscle tissue during the gestation.

DNA was isolated from 26 liver and 26 muscle tissue samples obtained at autopsy from miscarriages between 13<sup>th</sup> and 28<sup>th</sup> week of gestation. The mtDNA amount was analyzed by the real-time PCR method using SybrGreen I (Chromo4, Bio-Rad).

The significant positive correlations were found between the gestational age and the relative mtDNA amount in fetal liver tissue ( $r = 0.56$ ;  $p < 0.01$ ) and mtDNA amount in fetal muscle tissue ( $r = 0.61$ ;  $p < 0.01$ ), respectively.

In both fetal liver and muscle tissue, mtDNA content per cell was increasing with onward fetal development. These results are in accordance with the few of studies but there was never analysed as numerous set of tissue samples as in our study. Presently we analyze a relation among changes of the mtDNA content during prenatal development and the transcription activation of some mtDNA maintenance factors (NRF1, TFAM) in the same set of tissue samples.

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#### P06.183

##### Duplication analysis of the DMD gene by MLPA technique

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Duchenne and Becker muscular dystrophies (DMD/BMD) are the most common form of dystrophinopathies, with a reported incidence of 1:3500 and 1:18000 birth males, respectively. About 65% of DMD/BMD cases are attributable to large deletions of the DMD gene, whereas the remaining cases are caused by duplications or point mutations of the gene. The exact frequency of these two last types of mutations is not known. The detection of duplications in Duchenne (DMD)/ Becker muscular dystrophy (BMD) has long been overlooked. Recent techniques such as multiplex ligation-dependent probe amplification (MLPA) have simplified the detection of duplications.

We report here 20 duplications recently detected in BMD/DMD unrelated patients using MLPA technique. Although the reading frame rule could be applied in most patients, it was not applied in 2 DMD patients. Special care was taken with one patient presenting non contiguous rearrangements: **c.[2623\_2950dup; 11047\_11055del]**, a duplication of exons 21 and 22, and a deletion of exon 79. The majority of the duplications clustered towards 5' end of the gene.

The availability of new quantitative methods including MLPA has aroused interest in duplication detection analysis in DMD gene. The MLPA technique enables us to test all the 79 exons, allowing the detection of a significant number of new duplications. We report the duplications in patients in whom a previous molecular screening did not show any deletion.

#### P06.184

##### A novel MLPA technique for copy number analysis on small amounts of DNA

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Aim: The aim is to exploit the potential of MLPA in areas where DNA material is limited. The DNA concentration required in standard MLPA analysis is not attainable from dried blood spot samples (DBS) often used in neonatal screening programs. By redesigning the MLPA probes we attempt to perform MLPA analysis on small amounts of DNA.

Patients and methods: 7 patients with Congenital Adrenal Hyperplasia (CAH) were used in this study. DNA was extracted from both whole blood and DBS, and subjected to MLPA analysis using SALSA P050B probemix (normal and modified probes). Results were analysed using GeneMarker™ (Softgenetics) and Excel spreadsheet analysis.

Results: We found a 99.6% concordance of the results obtained with DNA extracted from whole blood compared with DNA from dried blood spot samples.

Conclusion: We demonstrate that MLPA reactions with modified probes are functional and reliable at very low DNA concentrations. This broadens the diagnostic perspectives of biobanks consisting of DBS allowing for CNV analysis in general and particularly testing for CAH.

This study is supported by the SAFE Network of Excellence

#### P06.185

##### Is SNPs in *MSX1* gene playing a role in the development of cleft palate (CP) or cleft lip/palate (CLP)?

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*MSX1* gene role in the development of cleft palate and cleft lip/palate and oligodontia has been confirmed in several studies. *MSX1* (member of muscle segment homeobox gene family) gene expression is related to transcription repression during embryogenesis and craniofacial development.

It was confirmed, that mutations in *MSX1* gene approximately in 2% can cause nonsyndromic CL/CLP. Results were replicated in studies involving different populations.

Objective of our study was to evaluate role of mutations and also SNPs in *MSX1* gene in Latvian nonsyndromic CP and CLP patients.

Materials and methods. DNA from 101 persons with CP or CLP was extracted from venous blood (CP- 21, CL/CLP-80). Promoter region, exons, introns boundaries and 3' UTR region were sequenced.

Results. One mutation 457G>C in exon 2 and 16 SNPs in *MSX1* gene was identified. Thirteen of them were described earlier and were commonly met in Caucasian populations, three of them were not observed in previous studies.

Interestingly two SNPs, who were localized in intron 1 451+(41-51)del and 3'UTR 3969 A>G showed higher frequencies in nonsyndromic CLP patients (0.41;0.38 respectively) than in CP patients (0.17;0.14). Conclusions. Considerable difference observed between SNPs in patients with CP and CLP, who had localised in the non-coding regions, raise suggestions about their possible role in the development of non-syndromic CLP.

#### P06.186

##### Association of C677T and A1298C polymorphisms in the Methylenetetrahydrofolate reductase (MTHFR) gene with cervical dysplasia in Yucatan, Mexico

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The influence of MTHFR activity on DNA methylation, synthesis and repair, presents MTHFR as a candidate cancer-predisposing gene.

Polymorphisms C677T and A1298C in MTHFR gene have been associated with a varying risk of cervical dysplasia. We evaluated the association of C677T and A1298C polymorphisms at the MTHFR locus with cervical intraepithelial neoplasia (CIN) or cervical cancer (CC) in Yucatan, Mexico, where CC is the first most common cancer. A case-control study was performed. Cases were 84 DNA samples of women with CIN or CC (47 CIN I, 14 CIN II, 7 CIN III, 9 invasive cervix cancer and 7 cervical cancer) and controls were 127 DNA samples of women who were negative to PAP. Polymorphisms were determined by PCR-RFLPs. Allele and genotype frequencies were compared between cases and controls in EpilInfo software (OR, IC 95%). Genotype frequencies in cases and controls were according to Hardy-Weinberg expectations ( $p>0.33$ ) for both polymorphisms. For C677T-MTHFR polymorphism, allele and genotype frequencies were not significantly different between cases and controls ( $p>0.05$ ), whereas for A1298C polymorphism, frequency of allele C was significantly different between cases and controls ( $P=0.0325$ ), suggesting an association of 1298C allele with a reduced risk to CIN or CC (OR 0.51, IC 0.25-0.98). Stratification according to phenotype showed that heterozygous genotype AC and allele C were associated only with CIN I,  $p=0.023$  and  $p=0.0180$ , respectively. Our results suggest that polymorphism A1298C in MTHFR gene is associated to CIN I as a protective factor to develop more severe dysplasias in Yucatan population.

#### P06.187

#### MTHFR gene polymorphisms and neural tube defects - the incidence in Slovak population.

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Neural tube defects (NTD) belong to the most debilitating birth anomalies. The geographical & historical differences in NTD incidence vary tremendously - from 8/1000 till 0,21/1000. Discovery of folic acid's preventive effect influenced the research of NTD genetic background focusing to the genes whose products take part in the folate metabolism. One of the most important enzymes involved is MTHFR - methylene-tetrahydrofolate reductase whose common termolabile variant C677T in homozygote state reduces MTHFR activity to 30-40% of norm. Lowered activity might play a crucial role in early embryonic development. That is why this polymorphism has been investigated in various NTD populations and in some of them the mutant variant has been proven to be a risk factor for failure of neural tube closure.

In Slovakia with average natality about 50 000 liveborn per year, there are annually 10-20 children born with NTD (0,28/1000) - mostly with meningomyelocele. When including stillborn and selective abortions, the number of NTD pregnancies is even higher (0,35-0,52/1000). To evaluate genetic risk of folate metabolism variations in our population, we investigated MTHFR gene polymorphisms C677T and A1298C in 91 Slovak children with NTD. The obtained results of genetic analyses are being compared with those from 300 newborns from Slovak population. Currently we got the results of C677T polymorphism analysis, which, however, did not show any significant difference in the prevalence of TT genotype or T allele between the patients and controls (OR = 1,22 [95% CI 0,5-2,9]; OR = 1,16 [CI 0,8-1,7] respectively).

#### P06.188

#### Use of a Genetic Isolate to Identify Rare MS Variants

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MS is a chronic inflammatory disease of the CNS with complex inheritance. The etiology of MS is poorly understood. Large case-control association studies primarily aim to identify common variants contributing to pathogenesis of common traits. To tackle rare alleles contributing to the molecular background of MS, alternative strategies are needed. One approach is to use special populations with a founder effect and isolation, where one could hypothesize allelic enrichment. Such a unique high-risk region for MS is the Southern Ostrobothnia in Finland, where both the prevalence and familial occurrence are exceptionally high. We have performed a high-resolution genome-wide SNP study using the Illumina HumanHap300 panel to monitor, which alleles are shared by 72 familial MS cases from this high-risk isolate. Genealogical studies showed that majority of these patients are distantly related. Genotype data of 68 IBS-matched Finns was used as controls. We first focused on the 45Mb region on 5p, for which we have shown linkage in Finnish MS-families. The haplotype analysis over this region revealed association with one haplotype, and the finding was validated in a larger study set from the high-risk region (OR 2.73,  $p=3.2\times 10^{-6}$ ). The identified risk-haplotype flanks two genes, C7 (complement component) and FLJ40243 (predicted protein). Suggestive association with C7-FLJ40243 alleles and MS was also seen in more heterogeneous populations. Interestingly, plasma C7 protein levels and total complement activity were observed to correlate with the risk-haplotype identified. Sequencing of the 31kb C7 region did not reveal an obvious causative variant. Sequencing of FLJ40243 is in progress.

#### P06.189

#### Genetic polymorphisms of IL12B and VDR in association with multiple sclerosis in russians

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We investigated genotypes and haplotypes frequencies for VDR and IL12B polymorphisms in case-control studies of MS in 92 Russian inhabitants of Tomsk region. Our results show significant differences of alleles and genotypes frequencies in the distribution of the polymorphism 1188A>C IL12B between MS patients and controls ( $p<0.01$  - for frequencies of alleles). The CC genotype frequency was three times higher in patients as compared to the controls (6.7% and 2.1%, respectively,  $p<0.01$ ). The allele C in MS patients was associated with shorter duration of the first remission ( $p = 0.04$ ) which was  $1.80\pm 0.28$  in those with the C allele and  $3.05\pm 0.56$  in other patients. An association of VDR T/t polymorphism with diseases were observed ( $p<0.05$ ). This marker was also associated with amount of eosinophils in MS patients ( $p<0.05$ ). On examination of VDR haplotype, including a B/b, F/f and T/t polymorphism, we reveal the difference between distribution in case and control ( $p=0.03$ ). We demonstrate that the haplotype Bft significantly higher observed at MS patients and btT at healthy person ( $p<0.05$ ). Received data allow suggests that the allele 1188 °C of gene IL12B and Bft haplotype of VDR gene could increase susceptibility to MS and have influence on clinical manifestation of diseases in Russians.

#### P06.190

#### Association of IL2RA/CD25 polymorphisms with susceptibility to multiple sclerosis

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Multiple sclerosis (MS) is a prototypic idiopathic neurodegenerative disease of the central nervous system (CNS) whose primary mechanism of injury is by inflammatory/autoimmune demyelination and, to variable degree, axonal damage. As a complex disease both environmental and genetic factors contribute to its pathogenesis. As part of an ongoing search for genes associated with MS we have tested the candidate gene IL2RA, which encodes the interleukin-2 receptor (IL2R)

alpha subunit of the high-affinity IL2R complex. Four polymorphisms located in this gene that could be implicated in functional modifications of IL2RA activity were genotyped by PCR/RFLP in a case-control study. We found an association of the *IL2RA* T allele, located at the 3' untranslated region (3'-UTR) of the gene, with increased susceptibility to MS (OR= 1.24, 95% C.I. = 1.01-1.53, P = 0.04). The International Multiple Sclerosis Genetics Consortium has also found an association between MS and alleles of the IL2RA by a Genomewide Study. Association of polymorphisms at the IL2RA in other diseases of autoimmune origin such as type I diabetes (T1D) and Graves' disease has also been reported. Functional studies will be required to uncover the causal SNPs and to determine what effects they have on the IL2RA protein and its role in MS.

#### P06.191

#### Association study of rs6897932 SNP in IL7Ra Gene with Multiple Sclerosis in Iranian population

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Multiple Sclerosis is an autoimmune, demyelinating disease with a strong genetic component, affecting 1/1000 people; mostly young adults; in western countries and about 35,000 in Iran. Previous genetic risk studies have failed to identify consistently linked genes outside of HLA on chromosome 6p. In recent studies allelic association of a polymorphism in the gene encoding Interleukin 7 receptor α chain (*IL7Ra*) reported as a significant risk factor in M.S. *IL7Ra* gene located on 5p13, spans 8 exons and encodes IL7Ra protein on B and T lymphocytes. Variable expression of IL7Ra splicing isoforms controls amount of IL7 absorption. From 14 SNPs detected in *IL7Ra* gene, rs6897932 in exon 6 and rs987107 in intron 6 seems to change the ratio of soluble and transmembrane isoforms of receptor. In this study we investigate the association of rs6897932 SNP by designed a Multiplex PCR in M.S patients and normal controls.

#### P06.192

#### Investigation of relationship between NDUFS1 gene and Multiple Sclerosis

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Multiple sclerosis (MS) is the most common inflammatory demyelination disease of the central nervous system. Preferential maternal transmission in familial cases and the occasional association of MS and LHON, suggests an involvement of mitochondrion (mtDNA, electron transport chain enzymes) in the MS etiology. Besides, in recent study, researchers found a relationship between biochemical defect in complex I enzyme activity and pathogenesis of MS.

Complex I is the largest complex of mitochondrial respiratory chain that contains 43 subunits. Thirty six subunits being encoded by nuclear and 7 subunits being encoded by mitochondrial DNA. As mutations in *NDUFS1* gene were reported previously in patients afflicted by complex I deficiency and also biochemical defect in complex I enzyme was found in Iranian MS patients, we encouraged to study relationship between *NDUFS1* gene and MS disease in Iranian MS patients. This gene is a nuclear gene encoding catalytic subunit of complex I enzyme. So we analyzed four exons of *NDUFS1* gene (8, 9, 15 & 19) that some mutations are identified in patients with complex I deficiency. We amplified these exons by PCR and screened them for finding any variations by SSCP method. Suspicious samples for mutation were sequenced. Finally, in our samples, we could not find any variation in these regions of *NDUFS1* gene. To identify the relation between *NDUFS1* gene and complex I with MS disease, further analyzes should be done on other exons of this gene and also other genes of complex I subunits.

#### P06.193

#### The role of the Protein Kinase C Alpha (PRKCA) gene in the predisposition to multiple sclerosis in the Italian population

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Multiple sclerosis (MS) is a chronic neurological disorder characterized by multicentric inflammation, demyelination, and axonal damage.

Besides the well-known HLA region, only 2 other loci (5p, 17q) have been so far linked and associated in at least 2 populations. The 17q region showed the most significant association with the Protein Kinase C Alpha (PRKCA) gene in Finns; this association was replicated in Canadian and UK populations, in which specific "risk haplotypes" were identified. Moreover, PRKCA transcript levels were shown to be higher in CD4+ mononuclear cells of MS patients carrying the risk haplotypes, suggesting a contribution of PRKCA regulatory mechanisms in the pathogenesis of MS.

In this study, we analyzed the role of PRKCA in the predisposition to MS in a cohort of 294 Italian cases and in an equal number of age- and sex-matched controls. An association analysis was performed genotyping 3 STR and 12 SNP markers covering the whole gene. A weak association (P=0.032) with the STR located in the PRKCA promoter was found. This region was further analysed by sequencing the first 424 bp of the promoter and the entire exon 1 of the gene in all MS cases and controls, but no genetic variants specific for MS cases were identified. Moreover, we identified a novel microRNA (hsa-mir-634) located in PRKCA intron 15, whose expression levels do not correlate with those of PRKCA. The potential role of hsa-mir-634 in the pathogenesis of MS is under investigation.

#### P06.194

#### Genome wide linkage scan for loci of musical aptitude in Finnish families: Evidence for a major locus at 4q22

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Background: Music perception and performance are comprehensive human cognitive functions and thus provide an excellent model system for studying human behaviour and brain function. However, the molecules involved in mediating music perception and performance are so far uncharacterized.

Objective and methods: We have applied molecular and statistical genetic approaches to unravel the biological background of music perception. We recruited 15 Finnish multigenerational families (with a total of 234 family members) via a nationwide search and defined the phenotype of all family members using three tests used in defining musical aptitude: a test for auditory structuring ability (Karma Music test; KMT) commonly used in Finland, and the Seashore pitch and time discrimination subtests (SP and ST respectively) used internationally. We calculated heritabilities and performed a genome wide variance components-based linkage scan using genotype data for 1000 microsatellite markers.

Results: The heritability estimates were 42% for KMT, 57% for SP, 21% for ST and 48% for the combined **music test scores**. Significant evidence of linkage was obtained on chromosome 4q22 (LOD 3.33) and suggestive evidence of linkage at 8q13-21 (LOD 2.29) with the combined **music** test scores using variance component (VC) linkage analyses. The major contribution for the 4q22 locus was obtained with KMT (LOD 2.91). Interestingly, a positive LOD score of 1.69 was shown at 18q, a region previously linked to dyslexia (DYX6), using combined **music test scores**. Conclusion: Our results show that there is a genetic contribution to musical aptitude that is likely to be regulated by several predisposing genes/variants.

**P06.195****Blind assessment of High Resolution DNA Melting Analysis as a tool for mutation screening**

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High resolution DNA melting analysis can be used to identify DNA variants in PCR amplicones. Using a LightScanner™ (Idaho Technologies) we have developed a single analysis protocol that allows the efficient screening of all PCR amplicones by high resolution DNA melting analysis to detect unknown DNA variants. In a blind assessment of our protocol we screened 14 different amplicones in 14 individuals and identified 23/23 sequence variants, distinguishing 100% of those where an individual was heterozygous for at least one site but only 57% of those individuals who were homozygous for a variant. In a direct comparison, analysis using the Wave® DHPLC system distinguished 96% of the heterozygous sequence changes and only 14% of the amplicones that differed by a homozygous sequence change. Our data suggests that high resolution DNA melting analysis using the LightScanner™ can be a highly sensitive mutation detection, which because of its low cost and high speed could potentially allow more comprehensive re-sequencing analysis of candidate genes.

**P06.196****Inter-laboratory diagnostic validation of Conformation Sensitive Capillary Electrophoresis.**

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Much of the work in diagnostic laboratories involves scanning for unknown mutations in particular regions of interest. Whilst this work can be done by sequencing, it is often faster and more economic to use an indirect pre-screen to identify fragments containing variations. Such methods need stringent validation to ensure diagnostic accuracy. Conformation Sensitive Capillary Electrophoresis (CSCE) is a rapid and sensitive method for indirect mutation scanning based on the principle that homoduplex and heteroduplex DNA have different mobilities when subjected to electrophoresis. We have performed a two phase diagnostic validation of this method across three laboratories. Phase I was used to define the performance characteristics of CSCE over a range of critical parameters including the nature of the mutation, fragment length and base composition, as well as a range of electrophoresis variables. Phase II comprised a blinded and randomised investigation of >400 different BRCA1 and BRCA2 variations to determine the diagnostic accuracy of both CSCE itself and a Bionumerics software plug-in used for the automated interpretation of heteroduplex patterns. We will report the findings of these studies and discuss their general applicability to diagnostic mutation scanning using CSCE.

**P06.197****MYO9B polymorphisms in multiple sclerosis**

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Multiple sclerosis (MS) is a complex disease manifesting as a chronic inflammation in the central nervous system. It is also commonly classified as an autoimmune disease (AID) and typical of many AIDs shows strong linkage and association to the HLA locus. In addition to HLA, AIDs are likely to share also other genetic risk factors. Recently, SNPs in the 3' region of the MYO9B gene have been reported to associate with celiac disease, inflammatory bowel disease, rheumatoid arthritis and systemic lupus erythematosus. The myosin IXB protein is an unconventional myosin with a Rho-GTPase activity. It is involved in actin cytoskeleton remodeling and is thereby a potential regulator of tight junctions and epithelial and endothelial permeability. High expression in leukocytes also suggests an immunological function. We tested the association of MYO9B variants with MS in four Northern European populations. Family-based association analyses (TDT, Gamete competition) using 18 SNPs covering MYO9B in 730 Finnish MS families showed no evidence for association. In order to increase the power to detect variants with smaller effect size we further genotyped 11 SNPs, 2 of which have previously shown association to other autoimmune diseases, in a set of 2511 MS patients and 2801 population controls from Finland, Denmark, Norway and Sweden. However, no association was observed in the case-control analysis, nor when stratifying for the previously identified genetic MS risk factors at HLA and PRKCA loci. Our results thereby do not support a major role for MYO9B variants in multiple sclerosis.

**P06.198****Functional analysis of missense mutations in the Myocyte Enhancer Factor 2A (MEF2A) gene do not support their causal role in the pathogenesis of myocardial infarction**

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Coronary artery disease (CAD) and its most severe complication myocardial infarction (MI), are the leading causes of death in Western countries. Mutations in the MEF2A gene, a member of the myocyte enhancer factor-2 (MEF2) family of MADS-box transcription factors, have been reported in patients with CAD/MI. In particular, a 21-bp deletion and 3 missense mutations were demonstrated to affect MEF2A activity either by reducing its transcriptional activity or by impairing its nuclear translocation. However, the association of MEF2A with CAD/MI was not confirmed by other studies.

In this work, we analyzed the role of MEF2A in the pathogenesis of MI in a large cohort (1785 males, 223 females) of Italian patients with premature MI (<45 y) and in an equal number of age- and sex-matched controls.

Firstly, the 21-bp-del was searched in the whole study cohort: it was found in 5 cases and was absent in controls ( $P=0.027$ ). Mutational screening of exon 8 (containing all known mutations) was performed in all 4016 individuals by DNA sequencing: 5 novel and 2 previously reported missense mutations were found. All identified mutations, either new or previously reported, were then functionally characterized by *in-vitro* expression in HeLa cells. Functional studies evidenced neither alterations in the transactivating properties (all mutants) nor defects in the nuclear localization (21-bp-del). An association analysis performed on 3 STRs at the MEF2A locus showed no significant association with premature MI. These data do not confirm the role of genetic variations in the MEF2A gene in the pathogenesis of MI.

**P06.199****Identification of a novel locus for shortsightedness (myopia) on chromosome 2q37 in three multigenerational Australian families**

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Myopia (short sightedness) is a complex trait influenced by both genetic and environmental factors. To date, fifteen myopia susceptibility loci (MYP1-15) have been identified but no definitive gene pinpointed. This study reports the identification of a novel locus for myopia located on chromosome 2q37 adjacent to, but not overlapping, MYP12. Three large multigenerational families with autosomal dominant myopia were recruited into this study. These families consist of 49 participants (35 affected) each of which has undergone a comprehensive ophthalmic examination. Individuals with other eye diseases that may affect vision such as glaucoma and keratoconus have been excluded. A genome-wide scan was performed using 400 microsatellite markers spaced an average of 10 cM apart. Using MERLIN, a multipoint parametric LOD score of 2.37 was calculated on chromosome 2q37. This LOD score increased to 3.43 with nonparametric analysis. The 1 LOD support interval initially suggested that this region may overlap with a known myopia susceptibility locus, MYP12. However, further fine mapping and haplotype analysis narrowed down the critical region to a 0.8cM region that no longer overlaps MYP12. Hence, a novel locus for myopia was identified on chromosome 2q37 between markers D2S1397 and D2S2968. Sequencing of all known and hypothetical genes in the region is underway in order to identify DNA sequence variants associated with myopia.

#### P06.200

#### Rapid Analysis of Myotonic Dystrophy 1 using Quantitative Fluorescent and Long PCR

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Diagnostics of myotonic dystrophy 1 (DM1) are based on the identification and determination of CTG repeat expansion in the DMPK-gene. This is usually done by Southern blot analysis - a time consuming and very laborious technique requiring high molecular weight DNA. Our study aimed at developing a highly sensitive, rapid and economical molecular analysis characterizing the CTG repeat region of DMPK-gene based on a two step PCR protocol. Therefore we analyzed 105 patients with the clinical diagnosis of myotonic dystrophy 1 derived from the DNA bank of the Institute of Human Genetics, University of Leipzig (Germany) who had already been tested by Southern blot analysis. 60 patients had one normal and one mutated allele of up to 2700 CTG repeats. 12 probands were homozygous for normal CTG repeat length. In the remainder (33 patients) two different normal alleles of up to 37 CTG repeats were present. Firstly, for the detection of alleles of up to 100 repeats quantitative fluorescent (QF) amplification with primers flanking the repeat region and 2 reference genes for standardization were used. With these methods it was possible to identify both homozygous and heterozygous DM1 alleles. Secondly, long PCR was just performed if a single wild type allele was detected by giving a QF-PCR-signal only half as intense. The results of using QF and long PCR indicate high accuracy in comparison to Southern blot analysis. We conclude that our new rapid analysis is reliable for genetic testing of DM1 patients.

#### P06.201

#### A case of nail patella syndrome with severe renal involvement in two sisters

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Nail-patella syndrome (NPS) is characterized by developmental defects of dorsal limb structures, nephropathy, normal tension glaucoma and sensorineural hearing impairment. NPS is a rare disorder with autosomal dominant mode of inheritance and is caused by heterozygous mutations in the transcription factor LMX1B. Proteinuria was described in 21% of individuals with NPS. Nevertheless, renal failure in a rare event.

A 38-year-old woman was admitted to our nephrology unit because of severe nephrotic syndrome. The diagnosis of NPS was established because of fingernail dysplasia, hypoplastic patellae, iliac horns and dislocation of the radial head. She underwent renal biopsy with the finding of proliferative glomerulonephritis at the age of 7 years. Her sister with NPS suffered under severe nephrotic syndrome and renal

insufficiency which lead to renal failure at the age of 13 years. Her father with NPS suffers only under mild proteinuria at the age of 70 years. All 8 exons of the LMX1B gene were amplified by polymerase chain reaction with described primers and then sequenced on an automatic fluorescent sequencer.

The missense mutation in exon 4 (c.599 G>A, p.Arg200Gln) of the homeodomain of the LMX1B gene was detected. This mutation was detected in the examined patient with renal failure as well as in her father with mild renal involvement. This missense mutation was already described in more families with NPS but only in individuals without proteinuria. The patient is nowadays dependent on peritoneal dialysis. To conclude, we describe a case of NPS with severe renal involvement.

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#### P06.202

#### Serum levels of some IgG and IgM type natural autoantibodies are differently regulated in carriers and non-carriers of HLA-DR16

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Natural autoantibodies (IgM or IgG type autoantibodies (Abs) present in the sera of most healthy individuals) are most important participants of the immune response. Little is known, however, on the genetic regulation of their plasma levels in human beings. Therefore we determined the concentrations of the IgM and IgG type antibodies against certain conserved self antigens (60 kD heat shock proteins (hsp60), citrate synthase (CITS), and chondroitine sulphate (CONS)) in the sera of 78 healthy individuals out of a family study with known alleles of several polymorphisms in the class I, class III and class II regions of main histocompatibility complex (MHC, HLA in humans). We found significantly lower serum levels of the IgM type CITS ( $p=0.029$ ) and CONS ( $p=0.026$ ) Abs in the carriers than non-carriers of the HLA-DR16 allele. Even stronger differences were found when levels of two Abs were considered. Frequency of the DR16 alleles were significantly higher in subjects with 1 or 2 low (in the lowest quartile) CITS/CONS Abs ( $p=0.002$ ), CITS-hsp60 Abs ( $p=0.001$ ) and CONS/hsp60 Abs ( $p=0.003$ ) as compared to those with normal Ab titers for both antigens. Abs against these antigens exhibited strong positive correlation ( $p=0.01$ ). By contrast, concentrations of IgG type anti-hsp60 Abs was significantly higher ( $P=0.008$ ) in the carriers than non-carriers of the HLA-DR16 allele. Similar differences were found when carriers and non-carriers of the HLA-DR16-DQ5 haplotype were considered. These novel observations indicate that not only induced, acquired but natural, inborn immune response as well is regulated by the MHC.

#### P06.203

#### Cytokine (TNF-alpha, TGF-beta1, IL-10, IFN-gamma, IL-6) genotyping in Turkish Children with nephrotic syndrome: A brief report

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Considering the recognized influence of cytokines in nephrotic syndrome (NS) development, the aim of this study was to investigate whether this disease may be associated with polymorphisms of the IL-6, IL-10, IFN-gamma, TGF-beta1 and TNF-alpha genes.

Forty-six children with NS, and 46 age-and sex-matched healthy controls were tested for 8 polymorphisms in 5 different genes. DNA was extracted from whole blood by standard salting out method. Cytokine genotyping was performed by polymerase chain reaction sequence-specific primer methods. The polymorphisms analyzed in the present study were IL-6 (-174 G/C), IL-10 (-1082 A/G, -819 T/C, -592 C/A), IFN-gamma (+874 A/T), TGF-beta1 (+10 T/C; +25 C/G) and TNF-alpha (-308 G/A).

The distribution of genotypes and allele frequencies were compared with the groups. No statistically significant differences were observed in allele distribution comparing NS and healthy groups ( $P>0.05$ ). The haplotypes of IL-10 and TGF-beta1 were compared in terms of their expressions and it was shown that the GCC GCC haplotypes of IL-10 had significantly decreased in the NS whereas there were no statistically significant differences in the haplotypes of TGF-beta1. The observed genotype counts were not deviated significantly from those expected according to the Hardy Weinberg Equilibrium ( $P>0.05$ ) except for IL-10 -1082 A/G polymorphism ( $P=0.048$ ).

We conclude that GCC GCC haplotypes of IL-10 gene polymorphisms (-1082 A/G, -819 T/C, -592 C/A) have been associated with NS in Turkish children. However, further studies with larger samples are needed to address the exact role of IL-10 in childhood NS.

#### P06.204

#### The C677T/A1298C Genotypes of *MTHFR* gene and Neural Tube Defects In Kazakhs

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This study determined the frequencies of *MTHFR* genotypes for 30 NTD cases, 84 their mothers and 150 unrelated healthy Kazakh adults for controls. The frequencies of combined C677T/A1298C genotypes among NTD cases were: CCaa - 6,7%, CCac - 6,7%, CCcc - 6,7%, CTaa - 10%, CTac - 46,7%, CTcc - 10%, TTaa - 10%, TTac - 3,3%. The frequencies of *MTHFR* genotypes among controls were: CCaa - 27,3%, CCac - 22%, CCcc - 7,3%, CTaa - 29,3%, CTac - 12%, CTcc - 0,7% , TTaa - 1,3%. Thus, the frequencies of CTac, CTcc, TTaa, TTac genotypes were higher than the frequencies of this genotypes in a controls ( $p<0.05$ ). The frequencies of *MTHFR* genotypes among the mothers of cases were: CCaa - 20,2%, CCac - 20,2%, CCcc - 8,3%, CTaa - 17,9%, CTac - 19%, CTcc - 6%, TTaa - 7,1%, TTac - 1,2%. The frequencies of this genotypes among controls were: CCaa - 28%, CCac - 21%, CCcc - 6%, CTaa - 30%, CTac - 13%, CTcc- 1% , TTaa - 1%. Mothers of Kazakh NTD cases were homozygous for the C677T mutations at a higher rate than their respective controls ( $p<0.05$ ). In summary, our study provides evidence that maternal C677T *MTHFR* homozygosity and CTac, CTcc, TTaa, TTac genotypes at the fetuses are a risk factor for NTD in Kazakh. The frequencies of TTaa and TTac genotypes were higher at the cases of high-level defects. The frequencies of CTac and CTcc genotypes were higher at the low level defects of spine.

#### P06.205

#### An association study of 45 folate related genes in spina bifida: involvement of Cubilin (*CUBN*) and tRNA aspartic acid methyltransferase 1 (*TRDMT1*)

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Spina bifida belongs to the neural tube defects, which are congenital malformations of the central nervous system with a prevalence of 0.5 to 12 per 1000 births around the world. In this study we aimed at identifying genes related to folate and its metabolic pathways that are involved in the aetiology of spina bifida. We selected 50 folate metabolism related genes and genotyped polymorphisms in those. Eighty-seven polymorphisms in 45 genes passed quality controls. Associations of those polymorphisms with spina bifida were investigated in a sample of 180 patients and 190 controls. The relation between polymorphisms that were associated with spina bifida risk and serum and red cell folate, vitamin B<sub>12</sub>, and homocysteine levels was evaluated in the controls. A polymorphism in *CUBN* was significantly associated with spina bifida after correction for multiple testing and was related to increased vitamin B<sub>12</sub> ( $P=0.039$ ) and red cell folate ( $P=0.001$ ). The *CUBN* gene encodes the intrinsic factor-cobalamin receptor (or Cubilin), a peripheral membrane protein which acts as a receptor for

intrinsic factor-vitamin B<sub>12</sub> complexes. Vitamin B<sub>12</sub> is an important co-factor in the folate metabolism and low B<sub>12</sub> status in mothers has been linked to neural tube defects in the offspring. Other interesting findings include nominal significant associations with polymorphisms in *TRDMT1*, *ALDH1L1*, *SARDH*, and *SLCA19A1* (*RFC1*). Our study points out interesting new candidate genes and functional pathways for further study, and confirms earlier findings. Neither of the genes *CUBN*, *TRDMT1*, *ALDH1L1* or *SARDH* have been investigated for association with spina bifida, before.

#### P06.206

#### Investigation of the putative functional effect of the 19bp Deletion polymorphism within Intron 1 of the Dihydrofolate Reductase (*DHFR*) gene

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*DHFR* is an important folate metabolising enzyme that catalyses the conversion of dihydrofolate to tetrahydrofolate. Folate genes are considered candidates for association with neural tube defects (NTDs) such as spina bifida due to the preventative effect of periconceptional maternal supplementation with folic acid. Investigation of an intronic 19bp deletion polymorphism within the *DHFR* gene found a significant protective effect in mothers of NTD cases when present in one (Relative Risk 0.59 (95%CI: 0.39-0.89),  $p= 0.01$ ) or two copies (Relative Risk 0.52 (95%CI: 0.32-0.86),  $p= 0.01$ ). Analysis of mRNA levels revealed a small increase in expression (~1.5 fold) associated with the 19bp intronic deletion polymorphism, but this was not significant (Parle-McDermott *et al.*, Am J Med Genet 143(11): 1174-1180 (2007)). We sought to further investigate the potential impact of the *DHFR* 19bp intronic deletion polymorphism on gene expression by employing a recombinant dual luciferase system in HEK293 cells. The results of these experiments showed that the 19bp deletion showed a modest increase in reporter gene expression in agreement with the mRNA data. It is proposed that the sequence of the 19bp deletion harbours an Sp2 binding site that acts as a repressor of transcription. Mobility shift assays are being employed to directly test whether this polymorphism results in loss of an Sp2 binding site.

#### P06.207

#### Mutations of *MFSD8* is a common cause of variant late-infantile neuronal ceroid lipofuscinosis

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The neuronal ceroid lipofuscinoses (NCLs), characterized by the accumulation of autofluorescent storage material mainly in neurons, are the most common neurodegenerative disorders of childhood. Six genes (*PPT1/CLN1*, *TPP1/CLN2*, *CLN5*, *CLN6*, *MFSD8/CLN7*, and *CLN8*) underlying late-onset NCL forms (LINCLs) have been identified so far. We analyzed the recently reported *MFSD8* gene for mutations in 79 patients, most of which had a clinical diagnosis of variant LINCL (vLINCL). Eight novel *MFSD8* mutations, including three missense, one nonsense, two splice-site, one deletion and one deletion/insertion mutation were identified in patients of various ethnic origins. Among them, a significant group of Roma Gypsy patients originating from the Czech Republic were shown to bear a mutation in *MFSD8*, possibly due to a founder effect. In one patient with a deletion/insertion mutation resulting in an in-frame amino acid substitution, the disease onset was later and the disease course less aggressive than in vLINCL. Our findings raise the total number of *MFSD8* mutations to 14, with the identified mutations evenly spaced within the gene and with the majority of families having private mutations. Our study confirms that *MFSD8* defects are not restricted to the Turkish population, as initially anticipated, but are a relatively common cause of vLINCL in different populations. *MFSD8* should be considered a diagnostic alternative in

vLINCL but also in NCL patients with later onset and a more protracted disease course.

#### P06.208

#### Linkage analysis in 238 ADHD-families identifies genome-wide significant signals on chromosomes 2q21.1 and 13q12.11 for neuropsychological measures

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ADHD linkage studies have not revealed consistent findings, suggesting alternative approaches to find genes involved in this disease. Endophenotypes, defined as heritable, continuously distributed traits that increase the risk for ADHD, may be more suitable for linkage analysis: Endophenotypes seem less genetically complex than clinical disease phenotypes. Moreover, they are often quantitative traits rather than dichotomous entities (like DSM diagnostic categories), hence the more powerful Quantitative Trait Loci (QTL) linkage analysis may be applied. Genome-wide linkage analysis was performed in the Dutch subsample of the International Multi-centre ADHD Genetics (IMAGE) study encompassing 238 DSM-IV combined type ADHD probands and their 112 affected and 195 non-affected siblings. Ten neuropsychological measures (cognitive and motor measures) previously shown to be candidate ADHD endophenotypes were used as quantitative traits. In addition, an overall component score of neuropsychological functioning was used, on which all ten individual measures related. A total number of 5,407 autosomal SNPs were used to run a multipoint regression-based linkage analysis. Two genome-wide significant linkage peaks were found, one for Motor Timing on chromosome 2q21.1 (LOD score: 3.944) and one for Digit Span on 13q12.11 (LOD score: 3.959). Eleven suggestive linkage peaks were found (LOD scores  $\geq 2$ ) on chromosomes 2p, 2q, 3p, 4q, 8q, 9p, 12p, 12q, 14q, 17q. The suggestive linkage signal of the component score at 2q14.3 (LOD score: 2.878) overlapped with the region linking to Motor Timing. In conclusion, our results suggest that neuropsychological candidate ADHD-endophenotypes may aid in the discovery of novel ADHD genes through linkage analysis

#### P06.209

#### NFATC4 gene polymorphism and aerobic performance in athletes

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Nuclear factor of activated T cells C4 gene (NFATC4) encodes transcription factor which regulates cardiac and skeletal muscle metabolism. The aim of the study was to investigate allelic distribution of NFATC4 gene Gly160Ala polymorphism in endurance-oriented athletes ( $n=549$ ) and controls ( $n=1057$ ), and to find interrelation between genotypes and physiological parameters in rowers ( $n=90$ ). Genotyping was performed by restriction fragment length polymorphism analysis. Physiological parameters were evaluated by PM 3 Rower Ergometer and MetaMax 3B Gas Analyzer. The frequency of NFATC4 Gly allele was significantly higher in athletes than in controls (51.5% vs. 43.7%;  $p<0.0001$ ), and increased with the growth of skills (sub-elite athletes: 36.7%-48.5%; highly elite athletes: 60.6%-62.5%). Furthermore, NFATC4 Gly allele was associated with high values of aerobic performance (when VO<sub>2max</sub> and AT in % of VO<sub>2max</sub> were measured). Thus, NFATC4 gene Gly160Ala polymorphism is associated with aerobic performance of athletes and plays an important role in sports selection.

#### P06.210

#### Pilot study on NIHL in a population of Portuguese noise-exposed workers

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Noise-induced hearing loss (NIHL) is one of the most important occupational diseases and, after presbycusis, the second most common form of sensorineural hearing loss. NIHL is a complex disorder, caused by an interaction between environmental and genetic factors. In contrast to the considerable amount of knowledge on environmental factors, almost nothing is currently known about the genetic basis of NIHL.

In humans, a few studies have been performed aiming at identifying an association between variants of relevant genes expressed in the inner ear and NIHL. Significant differences were recently detected between noise susceptible and noise resistant subjects for some variants of genes CDH23 and KCNE1.

In the pilot study here reported, we have analyzed a population of Portuguese noise-exposed workers. KCNE1 and CDH23 genes had been selected for genotyping of some SNPs, preferentially in coding regions, in order to be able to detect significant differences between noise susceptible and noise resistant individuals.

When individually analysed, significant statistic differences were found in three SNPs of exon 50 of CDH23 gene: rs4747193 (intrinsic SNP); rs4747194 and rs4747195 (nonsynonymous SNPs). However, it was not possible to identify a clear genetic association, when performing bivariate or multivariate analysis.

The lack of strong association between the variants considered in this study and NIHL can indicate that none of these polymorphisms has influence in the susceptibility to NIHL in the Portuguese population, or that their effect could not be detected in our sample due to its small dimension.

#### P06.211

#### Lack of important association between ND gene mutations and the risk of progression Norrie disease

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Background: Retinopathy of prematurity (ROP) is a retinal vascular disease which occurs in premature neonates with low birth weight and may lead to retinal detachment and blindness. Mutations in the Norrie disease (ND) gene have been reported as a risk factor of progression to advance stages in cases of ROP. We wanted to determine if any mutation in ND gene may play a role in progression of ROP to advance stages in Iranian infants.

Methods: Fifty Iranian premature neonates with ROP stage 3 and/or worse have enrolled in the study. After polymerase chain reaction (PCR) with three primer pairs and direct sequencing and all three exons and their flanking areas and all known ND gene mutation regions were evaluated for any mutation.

Results: A C15078A heterozygote mutation was found in the second intron of 90% of the cases but it has no effect on the structure or the production rate of the norrin protein, the product of ND gene. No other mutations causing changes in norrin protein were found.

Conclusions: Our findings from this case series suggest lack of important association between ND gene mutations and the risk of progression of the disease to advanced stages in patients with ROP.

#### P06.212

#### The interaction between gene polymorphisms and carbohydrate intake on metabolic profile in Russian athletes

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Genetic and nutritional factors interact together and modulate the plasma carbohydrate and lipid profile. The objective was to study whether carbohydrate intake modulates the association between NFATC4, PG-C1A, PPARA, PPARG, PPARD, TFAM, UCP2 and UCP3 gene varia-

tions and metabolic profile in Russian athletes. The study involved 33 male Russian sub-elite endurance-oriented athletes (road cyclists), who were randomly assigned to consume carbohydrates/minerals (CARB, n=17; 6% 200 ml drink "Olympia" (Estonia)) or placebo (CON, n=16; 200 ml pure water) for 20 d at 20th min from the end of evening training. Plasma concentrations of total cholesterol, glucose and resting lactate (La) were evaluated in the morning before and at the end of experiment. NFATC4 Ala160Gly, PGC1A Gly482Ser, PPARA G/C, PPARG Pro12Ala, PPARD +294T/C, TFAM Thr12Ser, UCP2 Ala55Val, UCP3 -55C/T gene polymorphisms were determined by PCR-RLFP. At base-line PPARA C allele carriers exhibited the highest values of La ( $P=0.008$ ); NFATC4 Ala ( $P=0.04$ ) and TFAM Ser ( $P=0.024$ ) alleles were associated with higher glucose concentrations. At the end of experiment PGC1A Ser ( $r=0.54$ ,  $P=0.03$ ), PPARG Pro ( $r=0.58$ ,  $P=0.019$ ) alleles were positively correlated with high values of La in CARB- and CON-groups, respectively, whilst PPARD C allele ( $r=0.47$ ,  $P=0.055$ ) was associated with higher total cholesterol levels in CARB-group. Furthermore, PPARG Pro allele carriers of CARB-group showed the greatest decrease in total cholesterol. Thus, polymorphisms of PGC1A, PPARG and PPARD genes (involved in carbohydrate and lipid metabolism) may interact with carbohydrate intake to modulate metabolic profile of endurance-oriented athletes.

#### P06.213

#### Cannabinoid type-1 receptor gene polymorphisms are associated with central obesity in a Southern Brazilian population

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#### P06.214

#### Association between haptoglobin phenotype and serotonin transporter gene polymorphism (5HTTVNTR) in the obesity inflammatory process

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Introduction: Obesity is a chronic disease associated with an increase of oxygen reactive species. BMI is related with immunologic changes resulting in a decrease of antigen tolerance and in a predominance of Th2 immunologic system profile. Haptoglobin is an acute phase protein. It has three genotypes 1.1, 2.1 and 2.2 that differ in their affinity to complex haemoglobin and that are involved in the modulation of immunological response. Genotype 1.1 is associated with a predominant Th2 response. Serotonin participates in Th1/Th2 balance as it regulates cytokines release. Their intra and extracellular concentrations are regulated by its transporter whose efficacy depends on its genetic polymorphisms.

Objective: Haptoglobin and 5HTTVNTR polymorphisms as risk factors for obesity.

Methods: 289 women were studied, 248 with overweight or obesity (BMI=30,19±3,74 Kg/m<sup>2</sup>; 47,31±12,63 years old) and 41 controls

(56,29±11,67 years old). 5HTTVNTR was amplified by PCR and haptoglobin phenotype by PAGE.

Results: Women caring allele 1 of Hp showed a higher risk for obesity (OR=2,270; IC 95% [1,011-5,098];  $p<0.05$ ) and the same was verified for allele 12 of 5HTTVNTR (OR=2,148; IC 95% [1,071-4,311];  $p<0.05$ ). The risk increases when these two alleles are associated (OR=3,659; IC 95% [1,426-9,390];  $p<0.01$ ).

Conclusion: An association between allele 1 of haptoglobin and allele 12 of 5HTTVNTR increases the predisposition for obesity. The fact that obese individuals show a higher risk for allergic diseases has been associated with a predominant Th2 response. It is in concordance with our results as both polymorphisms seem to change Th1/Th2 balance for a predominant humeral immunity.

#### P06.215

#### Association study of genetic polymorphisms in immunoresponse genes (*IL1b*, *IL1RN*, *TNFA*, *LTA*, *IL8*, *IL10*) in occupational chronic bronchitis

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The purpose of this study was to investigate the possible roles of the cytokines genes in the development of occupational chronic bronchitis. Polymorphisms in the genes encoding *IL1b*, *IL1RN*, *TNFA*, *LTA*, *IL8*, *IL10* were investigated in patients with occupational chronic bronchitis (N=122) and healthy workers (N=166) from Bashkortostan Republic. Tests for Hardy-Weinberg equilibrium among controls were conducted using observed genotype frequencies and a  $\chi^2$ -test with one degree of freedom. We also used chi-square tests to examine the association between genetic polymorphisms and individual susceptibility to occupational exposure. A value of  $p<0.05$  was considered as statistically significant. The frequencies of the *TNFA* (-308G/A) and *LTA* (252A/G), *IL1B* (3953C/T) and *IL1 RN* gene haplotypes were computed using the expectation-maximization algorithm with the EH software program. Odds ratios and 95% confidence intervals were calculated to estimate the individual risk of occupational chronic bronchitis.

No difference was found between patients and healthy controls in the distribution of genotypes and frequencies of alleles of the *TNFA* (-308 G/A), *LTA* (252A/G), *IL1B* (3953C/T), *IL1RN* (VNTR), *IL10* (-327C/A), *IL8* -251A/T gene polymorphisms. However, it was shown that the *IL1B*, *IL1RN* genes haplotypes frequency distribution patterns significantly differed between workers with occupational chronic bronchitis and healthy workers ( $\chi^2=9.31$ ,  $df=5$ ,  $p=0.03$ ). The *IL1B* 3953T/*IL1RN*\*2 haplotype was associated with higher risk of occupational chronic bronchitis (4.67% vs. 1.30%, in healthy workers;  $\chi^2=4.29$ ,  $p=0.04$ ; OR=3.73, 95%CI 1.06-14.29).

Our results indicate that the *IL1B* and *IL1RN* genes polymorphisms may play a role in pathogenesis of occupational chronic bronchitis.

#### P06.216

#### Common genetic polymorphisms in the human organic cation transporters 1, 2 and 3 and the pharmacokinetics of biguanide class drug metformin

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Organic cation transporter subtypes 1, 2 and 3 (OCT1, OCT2 and OCT3) mediate the cellular uptake of N,N-dimethylimidodicarbonimidic diamide (metformin) used as antidiabetic drug. Earlier, lost-of-function polymorphisms in the OCT1 gene were associated with reduced metformin efficacy in mice and humans.

We analyzed the effect of common genetic variants in the three genes on metformin pharmacokinetics in 103 healthy male Caucasians. Thirty functional or haplotype-tagging SNPs and one potential copy number variation were genotyped. Polymorphism-dependent and tissue-specific variation in gene expression was assessed by quantitative RT-PCR.

The reduction in the OCT1 activity due to one of the amino acid substitutions, R61C, G401S, 420del, or G465R, was associated with a higher renal clearance of metformin (means of 30.62, 33.14 and 37.14 l/h for carrier of two, one or none active OCT1 alleles respectively,  $P=0.04$ ). In addition, a haplotype in the promoter region of the OCT1 gene was associated with an increased renal clearance (30.96, 33.65 and 40.56 l/h for two, one or non haplotype carrier respectively,  $P=0.03$ ) and with

decreased gene expression in lymphoblastoid cell lines. No association was observed for the polymorphisms in the OCT2 and OCT3 genes. Additionally, previously suggested effect of the "silence" A411A polymorphism on the OCT3 splicing could not be validated in exon-trapping experiments.

In conclusion, genetic variants in humanOCT1 but not in OCT2 and OCT3 genes were associated with alternation in the metabolism of metformin. The best explanation of our data is that OCT1 is involved to some extend in tubular reabsorption of metformin.

#### P06.217

#### Interferon regulatory factor 6 (IRF6) variation contributes to oral clefts in South America

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Interferon regulatory factor 6 (IRF6) has been associated with non-syndromic (NS) oral-facial clefts (OFC). ECLAMC (Latin American Collaborative Study of Congenital Malformations) population that was included in the original IRF6 association study [Zuccherino et al., 2004] did not show association. Six SNPs within and in the boundaries of the IRF6 gene were genotyped to test for association with OFC in 304 trios of affected newborn patients and their parents. The ECLAMC samples were from Argentina, Bolivia, Brazil, Chile, Ecuador, Colombia, and Venezuela; there were 168 cases of isolated cleft lip with or without cleft palate (CL/P), 47 isolated cases with cleft palate only (CPO), and 73 cases with OFC and other defects. The most significant results were for SNPs rs 17015215 (VAL274I) in all OFC ( $p=0.0002$ ), and isolated CL/P ( $p=0.0004$ ), and rs 2013162 in isolated CPO ( $p=0.0009$ ). Suggestive results were also found for SNPs rs 861019 in all OFC ( $p=0.05$ ), CL/P (0.05), and CPO ( $p=0.02$ ), and rs 17015215 (VAL274I) ( $p=0.048$ ), and rs 2073487 ( $p=0.048$ ) in CPO. No association was found in the OFC group associated with other defects. Our results demonstrate that variation in IRF6 is a significant genetic contributor to OFC in South American populations.

#### P06.218

#### Allelic variants of IL1R1 and IL1RL2 genes associate with severe hand osteoarthritis

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**Objective.** In search for genes predisposing to osteoarthritis (OA), several genome wide scans have provided evidence for linkage on 2q. In this study we targeted a 470 kb region on 2q11.2 presenting the locus with most evidence for linkage to severe hand OA in our previous genome wide scan families.

**Methods.** We genotyped 32 single nucleotide polymorphisms (SNPs) in this 470 kb region comprising 6 genes belonging to the interleukin 1 superfamily. We monitored for association with individual SNPs and SNP haplotypes among severe bilateral hand OA cases ( $n=107$ ) and also end-stage bilateral primary knee OA cases ( $n=113$ ) and controls ( $n=436$ ).

**Results.** In a single SNP analysis with hand OA material, we found a significant association to a 174 kb region, covered by a single haplotype block, comprising genes *IL1R1* and *IL1RL2* ( $p$ -value=0.001).

Haplotype analysis provided further evidence for the *IL1R1* gene.

**Conclusion.** This study demonstrates a consistent association between hand OA and gene *IL1R1*. But since the associated SNPs are part of a wide LD block, we cannot unequivocally confirm which of the two genes explains the observed association. *IL1R1* and *IL1RL2* represent highly relevant candidate genes since they encode proteins that are known modulators of inflammatory processes associated with joint destruction.

#### P06.219

#### Association of polymorphisms and haplotypes in the 5' region of COL1A1 gene with BMD and osteoporotic fractures in Russian women from Volga-Ural region

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The collagen type I alpha 1 (COL1A1) gene is a strong candidate for susceptibility of osteoporosis. We analyzed the possible association of the COL1A1 genotypes and haplotypes, defined by -1997G/T, -1663indelT and *Spl* polymorphisms in 340 Russian postmenopausal women. Patients with chronic diseases and conditions which may potentially affect bone mass were excluded. The -1997G/T, -1663indelT and *Spl* polymorphisms were in strong linkage disequilibrium ( $D'$  = 0.66-0.95). The distribution of three common haplotypes in our population was as follow: -1997\*G/-1663\*insT/S - 62.2%, -1997\*T/-1663\*insT/S - 19.9%, -1997\*G/-1663\*delT/S - 14.9%. The *Spl* and -1663indelT polymorphisms were associated with BMD: homozygote carries of the *Spl*/S allele had increased BMD ( $p=0.035$  for FN-BMD;  $p=0.015$  for LS-BMD) and homozygotes for the -1663\*insT allele had higher values of BMD ( $p=0.017$  for FN-BMD;  $p=0.009$  for LS-BMD). There was a significant association between the -1997\*G/-1663\*delT/S haplotype and FN-BMD ( $p=0.01$ ) with reduced BMD values in homozygote carriers of the haplotype -1997\*G/-1663\*delT/S ( $p=0.023$  from carries of no copies of the haplotype by Tukey's post-test). We found an association of the -1663\*I\*D genotype ( $OR=1.7$ ; 95%CI 1.06-2.71;  $p=0.024$ ) and the -1663\*delT allele ( $OR=1.6$ ; 95%CI 1.09-2.4;  $p=0.015$ ) with osteoporotic fractures. The association of the -1997\*G/-1663\*delT/S haplotype with fractures was nominally significant ( $p=0.04$ ), but these did not reach the threshold for significance when multiple testing was considered. There was no significant association of the -1997G/T polymorphism with BMD or fractures in our population. Our results suggest that the COL1A1 gene is a susceptibility locus for osteoporosis in postmenopausal Russian women from Volga-Ural region.

#### P06.220

#### Epidemiological and genetic studies of otosclerosis with a new locus OTSC8 in tunisian population

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Otosclerosis is a very common hearing impairment among Caucasians with a prevalence of about 0.3-0.4% among white adults. In Tunisia, clinical otosclerosis has a prevalence of 0.4 to 0.8. Our aim is to perform an epidemiological, genetic and linkage analyses of otosclerosis in northern Tunisia.

Segregation analysis were performed and showed that the pattern of the disease is due to a rare dominant major gene with a high polygenic component.

Our study showed that in north-eastern areas of Tunisia, the sex ratio in probands with clinical otosclerosis being twice as often in women than in men. This probably suggests that an endocrine mechanism and/or biochemical factor are involved in disease aetiology. However, in north-western areas, there was no significant difference between the rates of otosclerosis between sexes.

Geographical distribution of affected subjects according to the ethnic origin of their parents showed that the areas with the highest concentration of affected individuals were urban or seaside areas. The frequency of otosclerosis was lower in rural areas and/or areas far from the seaside.

Genetic study was undertaken in some genealogies segregating autosomal dominant otosclerosis and linkage analysis showed that three families are linked to the known loci responsible of otosclerosis (OTSC1, OTSC2, and OTSC3). For the fourth family, the genome wide linkage analysis was performed and revealed a new otosclerosis locus named OTSC8.

The presence of a genetic factor associated with hormonal, biochemical or environmental factors, probably lead to variable expression of the otosclerosis according to age and sex.

**P06.221****High throughput mutation screening in patients with isolated respiratory chain complex I deficiency**

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Almost 50 causal variants in nuclear genes associated with OXPHOS disorders are known. Despite this success, in the majority of cases the molecular defects remain unknown. Most mitochondrial disorders are associated with three main categories of biochemical defects: respiratory chain complex I (RCC I), complex IV (RCC IV) and combined RCC I+RCC IV deficiencies. The number of genes potentially harbouring pathogenic mutations is very high. This situation calls for a technological improvement for carrying out the diagnosis of this group of disorders.

We deployed high-throughput protocols for genetic screening using high resolution melting point analysis (Idaho-Light-Scan). So far 96 samples have been analyzed in parallel followed by direct sequencing of those PCR products that display divergent melting curves. We have screened 50 genes (250 amplicons) coding for the subunits and assembly factors of RCC I. Causative mutations have been identified in 16% of patients. A single variant was identified in 30% of additional samples and in 54% of samples no mutations have been found yet.

**P06.222****Association of p53 polymorphism with recurrent pregnancy loss**

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The p53 tumour suppressor gene is a well-known factor regulating apoptosis in a wide variety of cells and tissues. Alterations in the p53 gene are among the most common genetic changes in human cancers. In addition, recent data provide evidence that p53 plays a critical role in mediating pregnancy by regulating steroid hormone activation. Several polymorphisms of the p53 tumour suppressor gene have been associated with recurrent pregnancy loss. We evaluated the hypothesis that polymorphisms in the p53 tumour suppressor gene in women may be associated with occurrence of repeated miscarriages.

The prevalence of a common polymorphism of the p53 tumour suppressor gene (Arg and Pro variants at codon 72) in 50 women with recurrent pregnancy loss compare with 50 normal women with at least two alive children as control group. For each patient, two p53 tumour suppressor alleles (Arg and Pro) were identified by using PCR-RFLP technique and genotypes were defined as Arg/Arg, Pro/Pro, or Arg/Pro. Statistical analysis was done by SPSS software and the p-values under 0.05 were considered to be statistically significant.

The homozygous Pro/Pro genotypes were found more often (27.5%) among women with recurrent abortion versus other group (21.9%).

It is concluded that P53 codon 72 polymorphism may serve as a susceptibility factor affecting the chances of recurrent pregnancy loss.

**P06.223****DNA polymorphisms in proinflammatory cytokines IL1B and IL6 are not associated to pain in spanish patients**

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The characterization of genes responsible for multifactorial diseases with genetic substrate such as pain, can be done through linkage analysis or association studies. Association studies can be performed with candidate genes.

In the case of pain, in addition to genes encoding proteins involved in neurotransmission, genes coding for inflammatory proteins can also play an important role. We have analysed allelic variants of genes encoding IL1B and IL6 proinflammatory proteins in order to determine whether variants of these genes may be associated with increased susceptibility to pain. We have studied 404 cases: 250 with neuropathic pain and 154 with inflammatory pain, in all of them the diagnosis

was accompanied by a study of levels of pain using EVA.

Our results show no relationship between allelic variants of studied genes, neuropathic pain, inflammatory pain, or with the levels of pain according to EVA. Therefore we conclude that pain is not associated with variants IL1B and IL6 genes

**P06.224****Alpha-synuclein gene duplication analysis in familial Italian Parkinson patients**

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Alfa-synuclein gene (SNCA) duplication or triplication was found in autosomal dominant Parkinson disease (PD). Our aim was to analyse SNCA multiplication in an Italian series of familial PD.

We examined SNCA multiplication in an Italian series of 134 unrelated Italian PD patients with at least one first degree relative affected. Our sample population derived from the "Human genetic bank of patients affected by Parkinson disease and parkinsonisms" (<http://parkinson.it/dnabank.htm>). We investigated the presence of SNCA multiplication using the MLPA approach (Kit "SALSA P51"). The kit also allow the detection of exon rearrangements in the *PKRN*, *PINK1*, *DJ1* genes and of two point mutations: p.G2019S (*LRRK2* gene) and p.A30P (*SNCA* gene). Each result was confirmed in two independent experiments. Up to now we performed MLPA assay on the first 58 familial PD patients.

This preliminary screening has identified 1 SNCA duplication positive patient, 1 heterozygous deleted allele in the *PINK1* gene (exon 6), 1 in the *PARK2* gene (exon 3-4) and 3 mutated alleles in the *LRRK2* gene (p.G2019S in exon 41).

The patient carrier of the SNCA duplication is a woman of 45 years of age affected by a classical PD. She developed depression and bradykinesia as first symptoms at 41 years. She responded to L-Dopa therapy and does not have cognitive decline. The mother developed PD at 45 years of age and died at 60 with dementia.

Our data indicate that SNCA duplication is present also in Italian population: approximately 1.5% (1/58) of familial PD patient.

**P06.225****Parkin mutation analysis in patients with sporadic early-onset Parkinson's Disease**

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Mutations in the *parkin* gene (PARK2) are responsible for about 50% of familial autosomal recessive early onset ( $\leq 45$  years) Parkinson's Disease (EOPD) and 10 to 20 % of sporadic EOPD. Recently, a novel *parkin* mutation, consisting of a deletion of the promoter and exon 1 of *parkin*, was described in a family with autosomal recessive EOPD and in an isolated case with EOPD. The aim of this study is to perform mutational analysis of the coding regions of the *parkin* gene in sporadic EOPD and subsequently to investigate whether rearrangements within both the shared promoter region and the *parkin* gene are present in the patients with only one or no mutations. A total of 53 index cases with sporadic EOPD and an age at onset  $\leq 45$  years from Southern Italy were screened for *parkin* mutation. DNA was extracted from peripheral blood using standard protocols and each exon of *parkin* was amplified and sequenced. Absolute quantification was performed by real time PCR 7900 HT-SDS. Among 53 patients screened for *parkin* mutations, 8 carried single heterozygous mutations, 4 had simple homozygous mutations, 1 was a compound heterozygous and 40 had no mutations. Gene dosage experiment failed to reveal an exonic rearrangement of

the *parkin* gene in patients with single heterozygous mutations. Our results show a substantial number of sporadic EOPD patients carrying single heterozygous mutations, suggesting that a second mutation could be localized in the promoter. In our study the gene dosage of core *parkin* promoter is still in progress.

#### P06.226

#### A functional G-463A polymorphism in the myeloperoxidase gene promoter and Parkinson's disease

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Epidemiological studies suggest that inflammation increases the risk of developing Parkinson's disease (PD) and experimental models of PD show that inflammatory oxidants modulate neuronal death. Myeloperoxidase (MPO) is an antimicrobial enzyme involved in the inflammatory process that produces reactive oxygen species. It is demonstrated that the levels of catalytically active myeloperoxidase are elevated in diseased brains and a functional G-463A polymorphism in the promoter of the MPO gene is associated with the risk of developing neurodegenerative disorders. There is biologic evidence implicating MPO in PD neurodegeneration process, but no data are currently available on the possible role of MPO polymorphism in the development of PD. Our study is the first to examine the association between MPO genotype and PD risk. We investigated the G-463A polymorphism in 233 PD cases and 100 controls. All patients were screened for this SNP by combination of PCR and RFLP analysis. We did not find significant differences in allele or genotype distribution between PD cases and controls ( $p=1$ ). Genetic findings show that the less common A allele decreases myeloperoxidase expression, apparently by destroying a binding site for the transcription factor. The reactive oxygen species play an important role in PD and individual susceptibility to PD may be modulated by the G-463A SNP. This work will be completed by increasing the number of the controls analysed to provide a more powered study. Our preliminary data suggest that G-463A MPO polymorphism is not a risk factor for PD nevertheless further studies in other populations are needed to confirm these results.

#### P06.227

#### Characterization and replication of a novel locus for late-onset Parkinson's disease detected in a genome-wide association study in an isolated population

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Genetically isolated populations have two major advantages above general populations when conducting genome-wide association studies: 1- more extended LD allows for using less markers and 2- less genetic heterogeneity, hence a higher relative risk of individual susceptibility alleles. We have performed a genome-wide association study in a young genetic isolate in Turkey for late onset Parkinson's disease (LOPD). This isolate exhibits increased prevalence of the disease suggesting that susceptibility alleles of relatively high risk are segregating in this homogeneous population. We used the Affymetrix 10K SNPChip to genotype 31 LOPD disease patients and 27 unrelated controls. Strong LD ( $r^2>0.8$ ) was commonly found up to approximately 150kb, hence the 10K SNPChip is sufficient to cover the entire genome in this isolate. Single SNP associations were carried out followed by a permutation test to determine the genome wide significance threshold. One SNP, rs1492592, was found to be significantly associated with LOPD ( $p=4\times 10^{-6}$ ). Finemapping a 1.1 Mb region surrounding the initial SNP has confirmed the association. Within 1.5 Mb of the locus, several interesting candidate genes are located, most notably GRIN3A and PPP3R2. A known limitation of using genetic isolates is that the loci detected in one population might not be of relevance in other isolates or the general population. Therefore we screened two additional genetic isolates and the general Dutch population for association with tagging SNPs covering six candidate genes. We replicated our locus by showing that multiple tagging SNPs are significantly associated with LOPD in the additional populations.

#### P06.228

#### ParkScreen: a Linkage Marker Panel for Parkinson's Disease

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**PURPOSE:** Parkinson's disease (PD) is a complex disease with both genetic and environmental susceptibility factors. We set out to develop a rapid inexpensive tool to aid in the characterization of known or novel loci that may contribute to PD in families. **METHODS:** We developed a genetic marker screening panel, ParkScreen, optimized for simultaneous marker amplification, to test families for linkage to known PD loci using only a few affected individuals. We also used ParkScreen for screening patients within genetically isolated homogenous populations. It was applied to three groups of apparently sporadic PD patients originating from different Italian Alpine valleys. **RESULTS:** Panel functionality was proven by detection of linkage to *PARK2* in a family with known *Parkin* mutations. Linkage to several known PD loci in the examined pedigrees was excluded, and suggestive linkage to *PARK8*, *PARK3*, and *PARK11* was found. For one family, linkage was excluded for all known loci, thus marking it as a candidate for further studies. **CONCLUSION:** ParkScreen is a useful, rapid, and inexpensive tool for assessing the involvement of known loci in familial PD. It allows the selection of families suitable for clinical follow-up and genotyping in order to identify specific mutations or novel genes.

#### P06.229

#### A genetic variant in PC-1 gene is associated with susceptibility to type 2 diabetes among Japanese population

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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 population. We previously found no evidence for association of K121Q polymorphisms with risk of type 2 diabetes in a Japanese population. This study for 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 their body mass index (BMI) status revealed no significant impact of the 3 polymorphisms and Q-delT-G haplotype on BMI. In conclusion our study indicated the potential role of an intronic polymorphism IVS20delT-11 with type 2 diabetes in Japanese.

#### P06.230

#### Searching for molecular markers of polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) affects about 10% of women in reproductive age. PCOS is recognized in the presence of at least two of the following symptoms: oligomenorrhea, hiperandrogenism, polycystic ovaries morphology. Disease results in infertility and increased risk of diabetes type 2, cardiovascular disease, endometrial cancer. Etiology of the syndrome remains unknown, although interaction of genetic and environmental factors is mostly discussed. Several meta-

bolic genes are probably involved in PCOS pathogenesis.

The aim of our study was to investigate whether there is a correlation between variants of genes: *IRS-1*, *IGF-2*, *SHBG*, *INS* and symptoms of PCOS in Polish patients.

Up-to-date 64 PCOS patients (diagnosed on the basis of criteria mentioned above) and 37 healthy controls were enrolled to our study.

We focused on four polymorphisms G972R (*IRS-1*), Apal-rs680A/G (*IGF-2*), TAAAn (*SHBG*) and VNTR classI/III (*INS*) tagged by -23Hph1-rs689 A/T.

Our results indicate that:

- prevalence of 972R variant is higher in PCOS group
- allele A of Apal is less frequent in the patient group compared with controls. On the contrary genotype A/A is more prevalent in patients group.
- VNTR classI/III distribution in both groups is nearly the same, whereas class III alleles homozygotes are more frequent among PCOS patients
- TAAAn - any association with neither PCOS nor its severity was found.

Our preliminary studies indicate, in consistent with literature, that variants of genes (*IRS-1*, *IGF-2*, *INS*) can be among the factors involved in PCOS pathogenesis. Nevertheless further analysis on larger cohort is performed.

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#### P06.231

#### Linking Genetic Susceptibility with clinical findings: a case study in periodontitis

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Diagnosis and hypothesis formation in clinical decision making (CDM) involves the integration of patient history, clinical and laboratory findings. Patient history incorporates genotypic information and carries genetic susceptibility towards disease manifestation. We present a hybrid multi-stage computational model which, in the first stage assesses a genetic susceptibility index (GSI) towards disease, in the second stage links GSI with phenotypic (clinical and laboratory) parameters and in the third stage generates comprehensible knowledge patterns that tie together genotypic and phenotypic information sources. The model integrates two inductive machine learning methods, namely: association rule mining (ARM) and decision tree learning (DTL) with behavioral communication theory. ARM is used to support formation of genotypic patterns which, are taken through a behavioral communication theory model to establish GSI both at the genotype and phenotype levels. DTL is included at the third stage to validate learned knowledge.

The model is demonstrated by means of a case study on periodontitis. Data are drawn from the warehouse of ACTA and incorporate genotypic and phenotypic data. Genotypic data include single nucleotide polymorphisms (SNP) and origin and clinical data include microbial values, life style habits, and age. The application of the model on the abovementioned data validates the proposed approach.

Presentation is cast in topic "Genetic analysis, linkage, and association" and documents a generic procedure, which can be used in any endeavor aimed at genotype - phenotypic data and knowledge integration.

Work reported was partially supported via the INFOBIOMED NoE, and to be also used in the GEN2PHEN European project.

#### P06.232

#### The GATC project: identification of novel genetic markers of cisplatin induced hearing-loss in children

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Adverse drug reactions (ADRs) are potentially life-threatening responses to medications. Children are at greater risk for ADRs, because >75% of approved drugs used in children are untested in pediat-

ric populations. Adverse drug reactions in pediatric oncology regimens are especially severe and account for 22% of all pediatric oncology hospital admissions. Cisplatin is one of the most effective anti-cancer agents for the treatment of solid tumors. However, a major dose-limiting toxicity of cisplatin is severe, permanent hearing loss. In previous studies the risk of developing cisplatin ototoxicity was increased with younger age and higher cumulative dose, but the contribution of genetic risk factors remains undetermined. We aimed to evaluate genetic markers predictive of hearing loss in children treated with cisplatin. DNA samples and detailed clinical information from cisplatin induced hearing-loss cases (n = 60) and drug-matched controls (n = 44) were collected through the GATC nation-wide ADR surveillance network. DNA samples were genotyped for a panel of 3072 single nucleotide polymorphisms (SNPs) in 220 genes. A significant genetic association with increased hearing loss risk was found for patients carrying variants in a key drug metabolizing enzyme (P-value=0.0001; OR=infinity). In addition, variants in known drug transporters were also associated with cisplatin ototoxicity (P<0.002; OR=3). Our results confirm findings that cisplatin-hearing loss is associated with a genetic susceptibility. The identification of genetic variants contributing to cisplatin ototoxicity is essential in the development of diagnostic markers to reduce the incidence of hearing loss, and make cisplatin treatment safer for children.

#### P06.233

#### The ability to become an elite endurance athlete depends on the carriage of high number of endurance-related alleles

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The objective was to evaluate the total contribution of CNB, NFATC4, PGC1A, PGC1B, TFAM, VEGF, UCP2, UCP3 gene alleles in defining predisposition to sports. The study involved 1580 Russian athletes and 1057 controls. CNB (calcineurin B) 5I/5D, NFATC4 (nuclear factor of activated T-cells, calcineurin-dependent 4) Ala160Gly, PGC1A (PPARgamma coactivator-1-alpha) Gly482Ser, PGC1B (PPARgamma coactivator-1-beta) Ala203Pro, TFAM (transcription factor A, mitochondrial) Thr12Ser, VEGF (vascular endothelial growth factor) G-634C, UCP2 (uncoupling protein 2) Ala55Val, UCP3 (uncoupling protein 3) -55C/T gene polymorphisms were determined by PCR-RLFP. We found that the frequency of endurance-related alleles (CNB I, NFATC4 Gly, PGC1A Gly, PGC1B Pro, TFAM Thr, VEGF C, UCP2 Val, UCP3 T) were significantly higher in Russian elite endurance-oriented athletes (n=351) compared to controls, both separately and cumulatively (45.6% vs. 37.4%, p<0.0001). Furthermore, 66.7% of highly elite endurance-oriented athletes (Olympic and World championship winners; n=12) were carriers of 8 to 12 endurance-related alleles (the others were carriers of 7 alleles), while there were only 18.1% of such persons in the control group (p<0.0001). Thus, the success in sports can be attributed to the carriage of high number of alleles associated with certain physical qualities.

#### P06.234

#### Screening of the PKD1 gene in Czech patients with autosomal dominant polycystic kidney disease

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Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease. The disease is caused by mutations of PKD1 (affecting roughly 85 % of ADPKD patients) and PKD2 (affecting roughly 14 % of ADPKD patients) genes, though in several ADPKD families the PKD1 and/or PKD2 linkage was not found. Mutation analysis of the PKD1 gene is complicated by the presence of highly homologous genomic duplications of the first two thirds of the gene.

The direct detection of mutations in the non-duplicated region of the PKD1 gene was performed in 78 nonrelated ADPKD patients. The direct detection of mutations in the whole PKD1 gene was performed in next 12 nonrelated ADPKD patients.

The duplicated region of the PKD1 gene was amplified by long range polymerase chain reaction (PCR) followed by nested PCR. Mutation screening was performed using denaturing gradient gel electrophoresis (DGGE), heteroduplex analysis (HA) and high-resolution melting (HRM). Suspected samples were then sequenced.

In the PKD1 gene we detected 30 germline mutations among 90 unrelated individuals; 24 mutations unique for Czech population. We identified 11 nonsense mutations, 13 missense mutations, 3 frameshift mutations, 2 deletion in-frame and 1 mutation in splice site. In the PKD1 gene we detected great numbers of different polymorphisms or unclassified variants.

Establishment of localisation and type of mutations and their genotype - phenotype correlation in ADPKD families will improve DNA diagnosis and could help to assess the clinical prognosis of ADPKD patients.

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#### P06.235

#### The association of metabotropic glutamate receptor subtype 8 (mGluR8) polymorphism with executive attention

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The glutamatergic signaling pathway represents candidate susceptibility system involved in mechanism of forming attention and working capacity. One of attractive candidate for study of molecular physiology of attention is metabotropic glutamate receptor subtype 8 (mGluR8). Expression of mGluR8 was observed in the olfactory system, the neocortex, the limbic cortex including the hippocampus and the amygdala. Electron microscopically, mGluR8 was largely observed on the axon terminals. Especially in several regions of the hippocampus it was found in the active zone of both asymmetrical and symmetrical synapses where mGluR8 may regulate glutamate release as an autoreceptor or GABA release heterosynaptically.

Influence of mGluR8 polymorphism located 29 bp after the termination codon (2756C/T) on executive attention was conducted on volunteers. 108 students of Moscow State University (mean age 20±2, girls = 61, boys = 47) were tested by Schulte tables. We found significant association of mGluR8 polymorphism with executive attention only in males. Carriers of T allele (TT,CT) implemented the test faster than CC group ( $p=0.02$ ).

#### P06.236

#### Q192R and L55M polymorphism of PON1 in patients with coronary heart disease (CHD) of different age and sex.

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Paraoxonase-1, a high-density lipoprotein-associated enzyme, is believed to protect low-density lipoproteins from oxidation and thus decrease the risk of CHD. The aim of this study was to investigate if paraoxonase-1 gene (PON1) Q192R and L55M polymorphisms influence the risk of CHD. 597 patients (438 men and 159 women) were included in our study: 227 men who survived myocardial infarction (MI) under the age of 45 (group I), 96 men with MI after 60 years (group II) and 115 healthy men (group III); 75 angiographically diagnosed CHD women (group IV) and 84 women without CHD (group V). All patients originated from Saint-Petersburg, Russia. Genotypes were determined by PCR-RFLP, paraoxonase activities were measured spectrophotometrically and standard enzymatic methods were used to determine levels of total cholesterol, triglycerides and HDL cholesterol on a spectrum analyzer. Statistical significance of differences between groups was assessed with  $\chi^2$ -test. We found that in group IV the frequency of 55M allele of PON1 was a statistically higher comparing to group V ( $p=0.029$ ). In group I amount of QR patients were significantly higher comparing to group IV ( $p=0.003$ ). In group II amount of QQ patients were significantly lower comparing to group IV ( $p=0.009$ ). There were no any differences in allele or genotype frequencies distributions be-

tween the groups of CHD men. Our results suggest that both Q192R and L55M PON1 polymorphisms play the role in risk of CHD. Furthermore, PON1 polymorphisms act in various ways in patients of different age and sex.

#### P06.237

#### The associations between PON1 55MM genotype and coronary artery disease and between the low HDL/LDL ratio values and the large arteries diseases

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The differences in the vascular diseases risk factor profiles, depending on the localization of the arterial diseases were analyzed in the series of 428 patients with coronary artery disease (CAD), 210 patients with aortoiliac occlusive disease (AIOD) and 255 cases of abdominal aortic aneurysm (AAA) - total 893 of vascular disease patients, as well as in 262 persons without symptoms of vascular disease. The SNPs of the genes related to the homocysteine metabolism: MTHFR 677 C>T, PON1-108C>T, L55M; as well as the lipoprotein profiles were analyzed in search for the functional differences predisposing either to the CAD or to the diseases of the large arteries. The SNPs were determined by PCR-RLFP methods.

In CAD group frequency of PON1 55MM genotypes (16,6%) was about 1,5-fold higher as compared to this either in AIOD+AAA group (10,8%,  $p<0,01$ ) or in control group (9,9%;  $p<0,01$ ), indicating the increased risk of CAD in persons with this genotype. In CAD group also the marginally higher frequencies of MTHFR 677TT and PON1 -108TT homozygotes were noted. On the other hand, the higher susceptibility to the large arteries diseases AIOD+AAA was found to be associated with the low values of HDL/LDL ratio ( $p<0,001$ ), the highly heritable parameter which may be related to the lower activity of paraoxonase 1. In summary, the PON1 55MM genotype and the low HDL/LDL ratio appear to be the vascular disease type specific risk factors for the CAD and the large arteries diseases (AIOD+AAA), respectively.

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#### P06.238

#### PPARG and PPARGC1A gene variants are associated with height in athletes

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PPARgamma nuclear receptor positively promotes adipogenesis and negatively regulates osteoblast differentiation, indicating that PPAR-gamma is a negative regulator of bone mass. The Ala12 variant of PPARG gene Pro12Ala polymorphism is associated with lower transcriptional activity, increased body mass index and height in humans. PPARGC1A has been identified as a transcriptional coactivator of PPARgamma. Carriers of the Ser482 allele have been reported to have lower levels of PPARGC1A by comparison with Gly482 allele homozygotes. Therefore, one could expect that the Gly482Ser polymorphism might affect height too. The aim of the study was to investigate an association of PPARG Pro12Ala and PPARGC1A Gly482Ser polymorphisms with height in Russian male rowers and speed skaters. The study involved 99 rowers (height - 191.1 (5.4) cm, weight - 86 (9.7) kg; aged 20-27) and 64 speed skaters (height - 179.6 (6) cm, weight - 74.9 (8.8) kg; aged 20-25). Rowers were divided into three groups: the highest group (195-204 cm), the middle group (189-194 cm) and the lowest group (182-188 cm). Gene polymorphisms were determined by PCR-RLFP. We found that the presence of the PPARG 12Ala allele was significantly associated with higher body height (Ala/Ala+Pro/Ala- 182.7 (4.9) cm vs. Pro/Pro - 178.7 (6.1) cm;  $P=0.023$ ) in speed skaters. The frequency of the PPARGC1A 482Ser allele was significantly higher in the highest group of rowers (33.3%), than in the middle (22.5%) and the lowest (18.8%) groups ( $P=0.032$ ). In conclusion, functional polymorphisms in PPARG and PPARGC1A genes may influence the growth of the skeleton in male athletes.

**P06.239****Susceptibility to preeclampsia in relation to ethnicity: multiple genes case-control study**

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Pathogenesis of preeclampsia suggests endothelial dysfunction during pregnancy. Considering that endothelial gene polymorphism may affect relevant protein production the aim of the study was to investigate whether genetic variability in endothelial dysfunction-related proteins contributes to individual and ethnic preeclampsia susceptibility differences.

317 women from Greece and Russia with normal pregnancy or uncomplicated preeclampsia participated in the case-control study. Polymorphisms of the TNF-alpha (-308G>A and -238G>A), NOS1 (AAT)n, NOS2 (CCTT)n, NOS3 (894G>T and 4a/4b), PAI-1 (4G/5G) and ACE (I/D) genes were determined by a PCR-based RFLP method.

Considering genetic heterogeneity between populations we carried out the comparison of allelic frequencies between controls from Russia and Greece and between patients from these countries. Analyzing results revealed that the frequencies of variants of NOS2, NOS3 and TNF-alpha genes differed between controls and patients according to ethnic origin. Our results show that it is incorrect to join samples from different countries if significant differences in allelic frequencies have been revealed.

According to the analysis of the polymorphisms independently and the development of preeclampsia, an association NOS3 4a/4b genotype (OR=2.18, 95%CI:1.07-4.45) was observed for Russian population. Severe preeclampsia was associated with TNF-alpha -308A allele (OR=5.07, 95%CI:1.10-23.48) in Greek population. When we investigated an interaction among these polymorphisms on the development of the preeclampsia, it was revealed that several combined genotypes were significant risk factors different in various populations.

The study of two populations has shown that preeclampsia is associated with misbalance in endothelial gene system, but genetic markers of disease vary in different countries.

**P06.240****The X-linked DIAPH2 gene is a risk-factor for Premature Ovarian Failure (POF) and accounts for female preponderance among POF patients offspring**

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Premature Ovarian Failure (POF) is a complex disorder, resulting in primary or secondary amenorrhea before the age of 45 and affecting 1% of women. Environmental factors have been shown to play a role in POF, but a strong genetic component is demonstrated by the prevalence of familial cases. The X chromosome was shown to be affected by structural rearrangements in several cases. Mendelian genes mutated in the disease are rare and the premutated allele at the FMR1 locus was shown to be a risk factor predisposing for the disease. These observations lead to the hypothesis of POF being a complex disorder caused by the additional effect of several risk factors.

The X-linked DIAPH2 gene was identified as interrupted by the breakpoint of a POF associated balanced translocation but its role in the pathogenesis of the disorder remains unclear as no causative mutations were found in a large number of cytogenetically normal patients. An association study was performed in a large cohort of POF patients and controls revealed the presence of a risk-haplotype significantly associated to the disorder. Moreover the analysis of the offspring sex-ratio in the POF cohort demonstrated a strong female preponderance mainly attributable to the risk-haplotype identified. Taken together these results demonstrated that the POF condition is preferentially due to additional risk-factors and thus with a multifactorial pattern of inheritance. The DIAPH2 gene is one of the susceptibility genes involved and its role in the process of oogenesis and ovulation will be further investigated.

**P06.241****Correlation of genotype/phenotype in primary Congenital Glaucoma patients from different ethnic groups of the Israeli population**

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Mutations in the CYP1B1 gene are responsible for more than 50% of primary congenital glaucoma (PCG) and mutations in the MYOC gene have also been associated with this disease. The OPTN gene is known to be associated with primary open-angle glaucoma and low-tension glaucoma. We noticed a different clinical presentation of PCG in our patients according to ethnicity. Our goal was to find a correlation between genotype and phenotype in people with PCG according to their ethnic origin. We screened the CYP1B1 gene in 86-individuals from 22-families of Israeli Moslem Arabs, Druze family and Ashkenazi and non-Ashkenazi Jewish, using the DHPLC apparatus. The screening revealed the cause for PCG in 11 of these 22-families. The mutations, includes a homozygous missense mutation R469W in exon-3 of the CYP1B1 gene in 4 different Druze and one Moslem families. Two Druze and two Moslem Arabs and one non-Ashkenazi families with the typical severe type of PCG were found to be compound heterozygous for two different DNA alterations. Screening the MYOC gene reviled in one of the non-Ashkenazi patient a heterozygous missense mutation and the patient was compound to another mutation in the CYP1B1 gene. No mutations were found in the OPTN gene in the families with no mutations in the CYP1B1 or MYOC. Establishing the genotype-phenotype correlations of PCG in our various ethnic backgrounds may add valuable knowledge for predicting the prognosis of the disease, for guiding therapeutic decision making and for genetic counseling of carriers of this cause of blindness in children.

**P06.242****Gene screening for primary lateral sclerosis on chromosome 4ptel-4p16.1 in a French-Canadian family**

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Primary lateral sclerosis (PLS) is a degenerative disease of upper motor neurons, characterized by a progressive spasticity resulting from the degeneration of cortical motor neurons which affects mainly the descending pathway of the corticospinal system. The lower extremities are first affected, followed by a spasticity of the trunk, of upper extremities and of bulbar muscles. A 550-marker whole genome scan was performed on a French-Canadian family with 12 individuals affected with PLS. This lead to the identification of a locus on chromosome 4 between the telomere and marker D4S2928 (4ptel-4p16.1). This is the first locus described for PLS. A maximum LOD score of 3.01 has been found for marker D4S2936. This region spans 10.2 megabase pairs and encompasses about 130 genes; 29 of those genes are clearly expressed in the brain. Five genes have already been screened without the detection of a coding mutation. We are currently screening the remaining genes in the candidate region. The identification of a gene responsible for PLS would help better understand motor neuron biology and pathology.

**P06.243****Haplotype associations of the MHC with psoriasis vulgaris in Russian patients**

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Psoriasis is a common inflammatory skin disease with a strong genetic component. Although eight psoriasis susceptibility loci (PSORS1-9) have been identified by genome-wide screening, linkage and association analysis have defined that MHC is the major genetic determinant related to psoriasis susceptibility. Haplotype associations of the MHC

with psoriasis vulgaris (PV) have been demonstrated in different racial or ethnic populations. To identify special haplotypes in Russian population that may contribute to the genetic susceptibility to PV, we investigated the distribution of the associated haplotypes in Russian cohort. 407 patients with PV and 418 controls were genotyped for *HLA-Cw6*, *HCR-C325T*, *HCR-A1911G* and *CDSN-C1243T* SNPs. The results showed: *HLA-Cw6* and *HCR-C325T*, *HLA-Cw6* and *HCR-A1911G* were in strong linkage disequilibrium (LD) ( $D'=0.67$ ,  $r^2=0.4$  in all subjects and  $D'=0.66$ ,  $r^2=0.37$  in patients;  $D'=0.87$ ,  $r^2=0.13$  in all subjects and  $D'=0.88$ ,  $r^2=0.16$  in patients, respectively), whereas LD was weaker between *HCR-C325T* and *CDSN-C1243T*; *HCR-A1911G* and *CDSN-C1243T* ( $D'=0.28$ ,  $r^2=0.024$  in all subjects and  $D'=0.13$ ,  $r^2=0.007$  in patients and  $D'=0.17$ ,  $r^2=0.019$  in all subjects and  $D'=0.10$ ,  $r^2=0.006$  in patients, respectively), suggesting a relative recombination hot-spot between *HCR* and *CDSN*. *Cw6/HCR-325\*T/HCR-1911\*G/CDSN-1243\*C* ( $\beta=1.5$ ,  $P<0.000001$ ), *Cw6/HCR-325\*T/HCR-1911\*G/CDSN-1243\*T* ( $\beta=2.6$ ,  $P<0.00005$ ) were identified as risk haplotypes for Russian population. In summary, our study of the Russian population suggest that a major psoriasis susceptibility gene resides in a region between *HLA-C* and *HCR* gene. Thus, the study of different populations will allow us to reduce the boundaries of the minimal PSORS1 region.

#### P06.244

#### Late cornified envelope (LCE) 3C and 3B genes deletion as a susceptibility factor for psoriasis

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Psoriasis is a common inflammatory skin disease with a prevalence of 2 to 3% in Caucasian population, characterized by abnormal keratinocyte differentiation and recruitment of inflammatory cells in the epidermis. Although it is generally accepted that environmental and genetic factors contribute to its etiology, the genetic susceptibility alleles of psoriasis remain poorly understood. By using comparative genomic hybridization (CGH) analysis we have identified a copy number variant (CNV) spanning 32 kb of chromosome 1q21 and mapping to the psoriasis susceptibility locus PSORS4. This CNV comprises two genes of the epidermal differentiation complex, LCE3C and LCE3B, both members of the late cornified envelope gene cluster. The absence of LCE3C and LCE3B is significantly associated ( $p = 0.0004$ ) with risk of psoriasis (OR = 3.0) in Spanish samples. The association was analysed in samples from The Netherlands and the US (combined allelic association of the three samples  $p < 10E-10$ ). The CNV-deleted allele is tagged by telomeric SNPs that showed epistatic effects with the HLA locus. Since LCE3C expression was also shown to be induced in psoriasis lesions of patients that do not have the LCE3C/LCE3B deletion, it can be postulated that on deletion chromosomes, other genes in the cluster could be expressed in a similar pattern. Preliminary analyses also suggest that the LCE3C/LCE3B CNV loss may have a positional effect on neighboring genes. Thus, loss of LCE3C/LCE3B or altered expression of LCE genes in genetically susceptible individuals harboring the deletion might lead to abnormal skin barrier formation and psoriasis susceptibility.

#### P06.245

#### Genomic gains and age at onset of psychosis

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**Background:** The recently discovered copy number variation of DNA segments is suggested to play a role in the development of complex disorders such as schizophrenia (Redon 2006, McC Carroll & Altshuler 2007). This abundant genomic variability (submicroscopic deletions and duplications) directly links disease phenotypes to gene dosage alteration. Although early onset forms of some neuropsychiatric disorders have been associated with genetic abnormalities, molecular mechanisms of early psychosis remain unclear. The aim of the present study was to study the relationship between copy number genomic variability and early onset psychosis.

**Methods:** We performed a comparative genomic hybridization (CGH) analysis in 20

Caucasian male patients with a first episode of schizophrenia-spectrum disorder characterised for: age at onset (age of first symptoms range: 13-30 years; assessed with the Symptom Onset in Schizophrenia Inventory, Perkins 2000), neurocognitive profile (WISC/WAIS) and premorbid adjustment (Premorbid Adjustment Scale, Cannon-Spoor 1982). Pooled DNA of 20 Caucasian healthy males, without psychiatric history in their first and second degree relatives, was used as reference in hybridizations. Array CGH analyses were performed using Agilent Human Genome Microarray 244K (genome-wide coverage).

**Results:** Genomic gains in chromosome 17q have been associated to early psychosis (childhood and adolescent onset) (Benjamini-Hochberg adjusted)  $p < 0.01$ .

**Conclusions:** Genomic variability in 17q chromosomal region seems to be underlying a significant proportion of illness early expression.

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#### P06.246

#### Is psychosis correlated with differential HSP90 expression?

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Endoplasmic reticulum (ER) has a clear role in stress response signaling mechanism. Clear association of heat shock protein with schizophrenia was presented by Kim et al (2001) indicating significantly higher levels of HSP90 variant among schizophrenia patients. Recently, the involvement of HSP90 variability in development of major psychotic disorders was renowned by studying of differential expression of this gene preferentially associated with its rs17034977 polymorphism in bipolar disorder patients (Kakiuchi et al. 2007). In this study, pathophysiological role of endoplasmic reticulum (ER) stress response signaling has been questioned for different subsets of patients with psychotic symptoms within schizophrenia, bipolar disorder and posttraumatic stress disorder. Association of specific HSP90B1 variant with psychosis was investigated.

#### P06.247

#### Gender-biased prevalence of the risk allele of the PTPN22 gene in patients with type 1 diabetes and Addison's disease

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The PTPN22 gene, encoding protein tyrosine phosphatase, a negative regulator in the T-cell activation and development, has been associated with susceptibility to several autoimmune diseases. The aim of this study was to investigate the association of PTPN22

G(-1123)C and C1858T polymorphisms with type 1 diabetes (T1D) and autoimmune Addison's disease (AAD) in the Polish population. A case-control study was performed with 215 T1D patients, 87 AAD individuals and 236 healthy controls. The PTPN22 G(-1123)C and C1858T SNP were genotyped using PCR-restriction fragment assay (Sacl and Rsal respectively). The CC genotype of G(-1123)C polymorphism was increased in AAD ( $p=0.030$ ) and (-1123)C was more prevalent in affected than in healthy males ( $p=0.003$ ). No significant difference was

found between T1D patients and controls. The 1858T allele presented an association with both T1D (OR=1.73, 95%CI 1.19-2.51,  $p=0.0035$ ) and AAD (OR=1.84, 95%CI 1.15-2.94,  $p=0.0099$ ). 1858T was associated with both diseases in males ( $p=0.022$  and  $p=0.008$ , respectively). No statistical difference in females was found. Stratification according to the presence of concomitant autoimmune disorders revealed an association of 1858T with both clinical forms of T1D and polyendocrine cases of AAD. G(-1123)C and C1858T were in linkage disequilibrium ( $D'=1.000$ ;  $r^2=0.5396$  for AAD, and  $D'=0.9818$ ;  $r^2=0.6104$  in T1D). No significant association between the *PTPN22* haplotypes and ADD was found. The haplotype including both mutant alleles was significantly more frequent in T1D than in controls ( $p=0.0033$ ). The results confirm the association of *PTPN22* C1858T polymorphism with T1D and AAD in Polish male population, while the G(-1123)C impact is less explicit.

#### P06.248

##### Mutation analysis of the PVRL1 gene in Caucasians with non-syndromic cleft lip/palate

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Non-syndromic cleft lip with or without cleft palate (CL/P, MIM 119530) is perhaps one of the most common major birth defects. It has been shown that homozygosity for a nonsense mutation of PVRL1, W185X, result in an autosomal recessive CL/P syndrome (CLPED1), whereas heterozygosity for the same mutation is associated with sporadic, non-syndromic CL/P in Venezuela. The aim of the study was to investigate further involvement of the PVRL1 gene in non-syndromic cleft lip and/or palate in North American and Australian Caucasian populations. Therefore, a mutation analysis of PVRL1 gene covering all isoforms were carried out in 216 North American and Australian Caucasian patients and 223 population-matched controls using Single Stranded both populations studied. Particularly two variants were more frequent in Caucasian nsCL/P patients than in Caucasian controls. One, an in-frame insertion at Glu442, was more frequent in Caucasian nsCL/P patients than in Caucasian controls though not significant and another, S447L (PVRL1 beta isoform specific one), was found to be significantly more frequent in non-syndromic CL/P in North American Caucasian patients versus controls ( $p=.032$ ), despite its failure to replicate in Australian population. These results suggest that PVRL1 may play a minor role in susceptibility to the occurrence of nsCL/P in Caucasian populations. It also suggests that the beta (HlgR) isoform might have a particular importance for craniofacial development based on this marginal association, and suggesting a possible genetic heterogeneity in the development of non-syndromic CL/P among the populations studied.

#### P06.249

##### Detecting associations in the presence of extreme allelic heterogeneity: Application to the rare variant common disease hypothesis

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Association studies are frequently utilized to map variants which are susceptibility loci for common diseases. Critical assumptions of this approach are that the disease is due to a common functional variant which is in strong linkage disequilibrium with genotyped SNP(s) and there is minimal allelic heterogeneity. If the rare variant common disease hypothesis holds, current association based methods will be underpowered due to allelic heterogeneity, low allele frequencies and poor correlation ( $r^2$ ) with tagSNPs. For common diseases where the underlying etiology is believed to involve extreme allelic heterogeneity, large scale candidate gene sequencing is currently underway to discover multiple causal rare variants. However, which methods are optimal for analyzing this type of data to detect associations is unknown. In this study, we analytically demonstrate that collapsing genotypes and rare variants across multiple loci is more powerful than multi-marker test (Hotelling's  $T^2$ ) and single marker test (Fisher exact test). Although bioinformatics tools are used to classify variants, variants can be misclassified and erroneously included or excluded from the analysis based upon predicted functionality. Collapsing methods are robust against misclassification of rare variants, but misclassification of common variants can lead to a substantial loss of power. For

the situation where it is suspected that both common and rare variants are involved in disease etiology a powerful and robust novel method was developed which combines collapsing and multilocus tests. It is also demonstrated empirically that for both collapsing and combined methods type I error is well controlled.

#### P06.250

##### New mutation in the IGF-I receptor (IGF1R) gene associated with intrauterine growth retardation

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**PATIENTS:** We have studied 55 spanish patients with short stature (<P3) associated with IUGR. We have also quantified plasma GH, IGF1 and IGFBP-3. The patients had normal or low levels of GH and high or normal levels of IGF1 and IGFBP-3. We have also studied 126 control samples.

**MATERIAL AND METHODS:** Genomic DNA was isolated from peripheral blood lymphocytes and amplified by PCR using primers that flank the coding regions of the 21 exons of the IGF1R gene.

Direct sequencing of the double stranded PCR fragments was performed (Abi Prism 310 Genetic Analyzer, Applied Biosystems).

**RESULTS:** In one patient with IUGR and short stature, we have found a mutation, in exon 7 in heterozygosis: Y487F. This mutation leads to a change in the aminoacid sequence. We have studied the IGF1R gene in this family and we have found this mutation present in the mother and the grand-mother also affected of short stature. This mutation was not found in the father, the aunt and the grand-mother aunt. We have not found this mutation in any of the 126 control samples studied.

**DISCUSSION:** The IGF1R gene is a tyrosine-kinase transmembrane receptor. The mutation Y487F changes a Tyrosine by a Phenylalanine, two aminoacid with a different polarity but with similar structure. This mutation in exon 7 should affect the extracellular domain,  $\alpha$  subunit. This region is responsive of the binding IGF1. This nucleotide change is not a SNP as it was not present in the controls

#### P06.251

##### Reelin gene variation in working memory performance

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Lack of convincing results in gene identification for psychiatric disorders has increased interest towards quantitative traits and endophenotypes, which provide more power for the data analysis and are potentially more closely related to underlying biology.

In our previous study, we replicated schizophrenia linkage on chromosome 7q21-32 in 352 Finnish families. A regional Reelin (RELN) gene on 7q22, encoding for Reelin glycoprotein involved in neuronal migration during the brain development, and contributing to synapse remodelling, crucial for cognitive abilities, showed strong association with an intragenic microsatellite marker (STR) and multiple cognitive traits in a subsample of 186 families with 618 neuropsychologically tested individuals.

Here, we utilized neuropsychological test data from 292 Finnish schizophrenia families with 923 tested individuals and 376 independent Finnish controls, and genotyped 96 RELN intragenic and flanking SNPs, two intragenic STRs, and one STR in RELN promoter. In the family sample, multiple SNPs associated with visual attention, visual working memory, learning, and executive functioning ( $p=0.006$  to  $0.0001$ ). Furthermore, we obtained strengthened evidence for association between the previous STR and especially verbal and visual working memory ( $p=0.007$  to  $0.00002$ ). Also multiple SNPs associated with positive symptoms of schizophrenia ( $p=0.005$ ). Interestingly, also in the control sample multiple SNPs associated with visual attention, visual working memory, information processing speed, and general abilities ( $p=0.004$

to 0.00008). The strongest signals emerged from the haploblock harboring the trait-associated STR. These data provide further evidence for involvement of RELN gene variations in cognitive functions.

#### P06.252

#### The risk of respiratory distress syndrome in neonates from Bashkortostan, Russia and gene polymorphisms of cytochrome P450 (CYP1A1, CYP1A2) and glutathione-S-transferase M1 (GSTM1)

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We have investigated association between polymorphisms of cytochrome P450 genes (CYP1A1 (A2455G, T3801C), CYP1A2 (A-163C, T-2467delT)), glutathione-S-transferase M1 (deletion GSTM1) and risk of developing respiratory distress syndrome (RDS) in neonates from Bashkortostan.

After birth molecular oxygen and free radicals influence on the neonates lung. They are potentially harmful to cellular components. Antioxidants provide protection against oxidative damage to proteins, lipids and DNA.

We used whole peripheral blood of 144 patients with RDS and umbilical cord blood of 217 healthy term neonates for the isolation of genomic DNA. It was used PCR amplification. We used chi-square tests to detect the association between genes polymorphisms and RDS babies. It was shown that the CYP1A1 (T3801C) gene genotypes frequency distribution patterns not significantly differ between patients with RDS and healthy neonates ( $\chi^2=1.92$ , df=2, P=0.382). In male infants the CYP1A1 TC genotype was associated with higher risk of RDS (38.1% in patients vs. 20.5% in healthy babies;  $\chi^2=6.8$ , P=0.009; OR=2.39, 95%CI 1.23-4.68). While, the CYP1A1 TT genotype had a protective effect (60.7% vs. 78.7%;  $\chi^2=7.0$  P=0.009; OR=0.41, 95%CI 0.22-0.81).

But at the same time we found no differences in the genotypes frequency distributions of the CYP1A1(A2455G), CYP1A2 genes within the patients and healthy groups.

We also didn't find any association of GSTM1 gene with RDS.

Our results showed that the polymorphisms in CYP1A1 may play a significant role in the development of RDS in male neonates.

#### P06.253

#### Genetic diagnosis of autosomal dominant and recessive Retinitis Pigmentosa using SNP high-throughput genotyping

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Retinitis Pigmentosa (RP), the major cause for blindness in the adults, is an extremely heterogeneous monogenic disorder which shows all mendelian types of inheritance. Up to now, more than 30 RP genes have been described, 17 of which are involved in autosomal dominant (adRP) and 20 in autosomal recessive (arRP) forms. However, many cases remain unassigned. Proper genetic diagnosis of the patients and potential carriers requires screening of all the candidates, but the high number of genes, with no major mutation sites, makes conventional mutation analysis time-consuming and costly for a small/medium sized laboratory. In addition, before undertaking the search for new RP genes, the already known candidates have to be ruled out. Taking into account these data and the fact that there is increasing evidence assigning the same candidate genes as responsible for distinct retinal dystrophies, we have designed an innovative time-cost effective strategy for cosegregation analysis of 39 RP and LCA genes by SNP genotyping on isolated families. This high-throughput strategy allows to discard the genes that do not cosegregate with the disease and highlights the remaining candidates. Subsequent mutational screening has allowed us to identify the causative mutations for several families. This type of analysis is very powerful in the arRP forms, particularly when the family is consanguineous. For adRP families, the analysis of several affected siblings is usually informative enough to discard most of the candidates. This approach can also be successfully applied to diseases with high genetic heterogeneity.

#### P06.254

#### Genomewide Linkage validation of the RP25 locus and Molecular Evaluation of Forty three Candidate Genes

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RP25locus (Chr.6:56.02-89.78Mb) was identified as the genetic cause in 14% of autosomal recessive Retinitis Pigmentosa (RP) in Spain. Linkage analysis has also reported the presence of this locus in Pakistani and Chinese families.

Our objective was: to confirm the initial findings of linkage to RP25locus by genomewide linkage analysis; mutation screening of genes from the RP25 region in 6 Spanish families with autosomal recessive RP (arRP) linked to this locus; analysis of new arRP families by microsatellite markers spanning the chromosome 6p12.1q15.

The data obtained using the 10KGeneChip-Mapping array have confirmed the original evidence of linkage to only the RP25locus in the three families with no linkage to any other region in the genome. Targeted linkage analysis of 18 newly ascertained arRP families led to the identification of five additional Spanish families also linked to RP25locus.

Bioinformatics analysis of the RP25 region indicated in excess of 111 genes with many showing retinal expression. 43 candidate genes were selected on the basis of their function, tissue expression pattern and/or the genetic data.

The direct sequence analysis from these genes (38.7%) led to the identification of 244 Single Nucleotide Polymorphisms, of which 76 were novel.

In conclusion, the fact that none of the observed variants were pathogenic exclude the screened genes as disease causing in these families. However, we could not rule out these genes as good candidates for other retinal degenerations mapping to the same chromosomal region.

These results support and validate the high prevalence of RP25 in the Spanish population.

#### P06.255

#### Retinoblastoma and microdeletion syndrome: identification of PCDH8 as a candidate for developmental delay

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Retinoblastoma (RB), the most common pediatric intraocular neoplasm, results from inactivation of both alleles of the RB1 gene, located in 13q14.2. Whole germline RB1 gene deletions represent 6 % of RB1 mutational spectrum. When the deletion involves the RB1 flanking regions it also causes a variable degree of mental retardation and several dysmorphic abnormalities.

In order to refine the role of chromosomal regions adjacent to RB1 in mental retardation and developmental delay, we decided to map the breakpoints in seven RB patients harbouring a whole gene deletion. Five of these patients presented no associated clinical abnormality, one presented a mild developmental delay with epileptic seizures, and one presented developmental delay with facial dysmorphisms.

To precisely map the deletion breakpoints we designed a 385 000 oligo-custom array (Nimblegen) focusing on RB1 and its flanking regions (34-74Mb). We also used MP/LC, a multiplex semi quantitative PCR assay running on a DHPLC platform to further narrow the breakpoint regions. Then PCRs spanning the breakpoints were performed and sequenced which allowed the deletion breakpoints to be defined at the nucleotide level.

We compared the deleted intervals between RB-only patients and RB patients with developmental delay and define a 3Mb critical interval that includes a good candidate, protocadherin 8 (PCDH8). PCDH8 is thought to function in signalling pathways and cell adhesion in a cen-

tral nervous system-specific manner.

Further studies are now needed to refine the critical interval and to check putative involvement of PCDH8 in developmental delay.

#### P06.256

#### A functional heme oxygenase-1 promoter polymorphism confers susceptibility to rheumatoid arthritis

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**Objective:** To investigate the role of heme oxygenase-1 (HO-1) gene as a novel functional candidate gene for rheumatoid arthritis (RA).

**Methods:** We carried out a case-control study including 736 RA patients and 846 healthy controls of Spanish Caucasian origin. Two putative functional HO-1 promoter polymorphisms, a (GT)n microsatellite and a -413 T/A single nucleotide polymorphism (SNP), were selected as genetic markers and genotyped using PCR-based methods. In addition, the intracellular expression of HO-1 was determined in healthy individuals with different (GT)n genotypes.

**Results:** The distribution of HO-1 (GT)n alleles (Short [S] ≤ 25 GT repeats and long [L]> 25 GT repeats) revealed a significant protective effect of S (GT)n alleles ( $P = 0.019$ ; OR 0.8, [95 % CI 0.7-0.9]) and SS (GT)n genotype ( $P = 0.002$ ; OR 0.4, [95 % CI 0.6-0.9]). In contrast, the -413 HO-1 promoter SNP did not yield any statistically significant deviation between RA patients and controls considering either allelic or genotypic frequencies. The haplotype analysis showed a strong protective effect of the S-A haplotype ( $P = 7E-07$ ,  $P_c = 0.000003$ ; OR 0.4 [95 % CI: 0.3-0.6]), whereas the L-A haplotype showed the opposite tendency ( $P = 0.008$ ,  $P_c = 0.03$  OR 1.2 [95 % CI 1.1-1.4]). In addition, we demonstrate that individuals carrying the SS (GT)n genotype show a significantly higher percentage of HO-1 expression than LL homozygous individuals ( $P=0.0003$ ).

**Conclusion:** In this study we identified the HO-1 (GT)n microsatellite as a new genetic marker involved in RA genetics in our population.

#### P06.257

#### STAT4 gene and the risk of Rheumatoid Arthritis in the Tunisian population

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The STAT4 (Signal transducer and activator of transcription 4) gene encodes a protein that plays an important role in the regulation and activation of certain cells of the immune system. In order to search an association of STAT4 rs7574865 T allele in the genetic predisposition to RA, we have studied 140 Tunisian patients affected with RA and 200 healthy controls. DNAs genotyping was carried out with a Taq-Man 5' allelic discrimination assay on an ABI 7500 real time PCR machine (assay: C\_29882391\_10) and data were analyzed by  $\chi^2$ -test, Genotype relative risk and Odds Ratio with 95% confidence interval. Our results showed that the T allele and the T/T genotype were more frequent in RA patients compared to controls ( $p= 0.008$ ;  $p= 0.003$ , respectively) (OR = 0.6; CI = [0.41-0.88]). We next examined patients RA subgroups according to clinical and immunological data for association with STAT4. A significant association of both T allele and T/T genotype were found in patients presented erosion ( $p=0.003$  and  $p=0.006$ ). A genotypic association was observed according to the presence of nodules ( $p=0.007$ ). Moreover, an allelic association were found when patients were stratified according to the presence of rheumatoid factor antibody (RF) ( $p=0.015$ ) as well as the presence of another autoimmune disease ( $p=0.021$ ). However, no significant differences in allele and genotype frequencies of rs7574865 were detected with anti-cyclic peptides antibodies (ACPA) positive RA patients ( $p=0.1$  and  $p=0.13$  respectively). These results support the involvement of STAT4 gene in the genetic susceptibility to RA in the Tunisian population.

#### P06.258

#### Specific neuromuscular diseases in the Roma population living in Hungary

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Interest in the unique genetic background of the Gypsy population led to the discovery of novel disorders in Bulgaria such as Hereditary Motor and Sensory Neuropathy type Loom (HMSNL) and type Russe (HMSNR) and the Congenital Cataracts Facial Dimorphism (CCFDN) syndrome. Studies across Europe characterized unique founder mutations in disorders such as Limb-Girdle Muscular Dystrophy 2C (LGMD2C), Congenital Myasthenia Syndrome (CMS) and Spinal Muscular Atrophy (SMA).

Our group has recently investigated affected Hungarian Gypsy families. Large experience was obtained with CMS, as 43 Gypsy families with 65 affected and 90 unaffected family members were analyzed. In 63 patients the 1267delG founder mutation has been detected in homozygous form in the CHRNE gene, whilst two independent patients showed compound heterozygosity of the frame shifting founder mutation and a missense mutation. Out of the other specific neuromuscular diseases three families showed HSMNL with homozygous R148X mutation in the NDGR1 gene and another family was linked to 10q22, the candidate region of HSMNR. Two patients were genetically diagnosed as having CCFDN with homozygous mutation IVS6+389C→T in the CTPD1 gene. Four patients were found to be affected by LGMD2C where the gamma-sarcoglycan gene carried the C283Y founder mutation in homozygous form.

In Hungary, 8% of the population is represented by Romans. Therefore, the exact knowledge of the frequent disorders, with the nature and prevalence of the founder mutations and the carrier frequency is required. Precise genetic diagnosis, carrier testing and genetic counseling programs should be offered for this population at risk.

#### P06.259

#### Integration of omic data in identification of sarcoidosis candidate genes

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Integration of genomic and transcriptomic information together with linkage analysis data allows for the identification of the genetic background of complex multifactorial diseases. This approach was performed in our search for possible candidate genes for sarcoidosis, combined with literature-based discovery knowledge.

A comprehensive search of Pubmed database for available transcriptomic, proteomic and genomic scan data for sarcoidosis has been performed. Additional information on possible candidate genes has been obtained by using BITOLA software, itself enabling advanced literature-based discovery searches of the Pubmed database. After careful inspection of discovered candidate genes, the most plausible candidates were selected for further analyses. Additionally, after identification of informative polymorphisms, the association of the candidate genes with the disease was investigated. In our previous studies we found a statistically significant association of PPARG and PPARGC1A polymorphisms with sarcoidosis.

By using *omic* approach we have identified new genes as potential gene candidates for sarcoidosis: Glutathione S-transferasePi (GSTP1), Endothelin-1 (EDN1), 25-(OH)-Vitamin D<sub>3</sub>-1α-hydroxylase (CYP27B1), Osteopontin (SPP1).

In our study on 105 sarcoidosis patients and 100 controls, the preliminary results did not show evidence of significant association of allelic variants of single polymorphism in GSTP1, EDN1, SPP1, CYP27B1 gene with susceptibility to sarcoidosis. Further studies on larger sample of patients and multiple polymorphism/gene molecular analyses are currently in progress.

**P06.260****Clinical and genetic analysis of a four-generation family with autosomal dominant cerebellar ataxia**

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Autosomal dominant cerebellar ataxias (SCAs) are a heterogeneous group of neurodegenerative disorders characterized by imbalance, dysarthria, progressive gait and limb ataxia which are variably associated with other neurological signs. There are many distinct loci associated to Mendelian forms of SCA, but only 15 genes have been identified (SCA 1-8, 10, 12-14, 17, 27 and DRPLA). Molecular genetic studies have shown that SCAs are caused by abnormal repeat expansions and that the age at onset is inversely correlated with repeat length. In the present study we conducted a genetic analysis of a SCA family from southern Italy, that included 15 affected members over four generations. The mean age at onset was 34 years with a strong evidence of anticipation across generations. We performed a mutational analysis searching for the most common SCA mutations and a linkage analysis for known genetic loci. The LOD score values were calculated using the LINKAGE program package, assuming an autosomal dominant inheritance and a disease frequency of 1 to 100,000. Penetrance in each subject was assigned to five liability classes determined from the age at onset. Pathological repeat expansions in the SCA 1, 2, 3, 6, 7, 8, 12, 17 and DRPLA genes were excluded. In addition, there was no evidence of linkage to SCA5 and SCA14 loci. We are now performing linkage analysis of remaining SCA known loci. The lack of any association will lead a full genome wide scan in order to identify the disease-related locus.

**P06.261****Association analysis of 42 SNPs in the TSNAZ and DISC1 genes in schizophrenia and bipolar affective disorder in the Polish population**

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Linkage between schizophrenia and chromosome 1q42.2 markers has been reported independently in different populations worldwide. This region contains the gene TSNAZ and Disrupted in Schizophrenia 1 (DISC1) which had been found to be disrupted by a balanced translocation and to co-segregate with schizophrenia in a large Scottish pedigree. Association studies investigating DISC1 single nucleotide polymorphisms (SNPs) in a number of samples were promising but not yet compelling.

In the present study, we aimed at investigating the possible contribution of TSNAZ and DISC1 variants on the pathogenesis of SCH and bipolar affective disorder.

We investigated 42 SNPs, on the basis of previous association findings, using MALDI-TOF mass spectrometry-based SNP genotyping (Sequenom's iPLEX technology). The study sample comprised 501 DSM-IV diagnosed patients with schizophrenia, 418 DSM-IV diagnosed patients with bipolar affective disorder and 530 controls - all from the Polish population.

For single marker analysis DISC1 rs1615409 was associated with schizophrenia at the allelic ( $p=0.001$ ) and genotypic ( $p=0.002$ ) level. TSNAZ rs1411776 showed an association with bipolar disorder and schizophrenia at the allelic ( $p=0.015$ , and  $p=0.053$  respectively), and genotypic level ( $p=0.038$  for bipolar disorder).

Our results provide evidence for the contribution of rs1615409 to schizophrenia susceptibility (particularly in females -  $p=0.0004$ ), and modest evidence for an involvement of rs1411776 in the predisposition

to both studied diseases. However, only one SNP association survived correction for multiple testing - for rs1615409 in schizophrenia. More detailed analyses, including haplotype and subgrouping according to age at onset and family loading are currently underway and will be presented.

**P06.262****A common haplotype of DRD3 affected by recent positive selection is associated with protection from schizophrenia**

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Recently, two independent reports identified association between haplotypes of the dopamine receptor (DRD3) gene and schizophrenia using different sets of tagSNPs. One of the reports suggests the existence of a common protective haplotype. A case-control association study was conducted in 273 schizophrenic patients and 512 controls from NE Spain. The characterization of the linkage disequilibrium patterns between tagSNPs of the previous reports was determined to allow for pooled analysis, comprising a total of 794 cases and 1078 controls. Searching for natural selection was done with tests based on extended linkage disequilibrium around specific haplotypes. The pooled analysis gives a strong statistical support for the existence of the protective haplotype (Mantel-Haenszel chi-square  $P$  value = 0.00227). This haplotype is linked to another haplotype at the locus, characterized by the Ser variant at the non-synonymous SNP rs6280, who increased its frequency recently by natural selection. Therefore, we can conclude that there is a DRD3 schizophrenia protective haplotype that has reached intermediate frequency due to the action of selection on a linked functional polymorphism. This finding reveals that natural selection may play a role in the existence of common alleles of susceptibility to schizophrenia, suggesting a new approach to find susceptibility loci.

**P06.263****Recent adaptive selection at MAOB and ancestral susceptibility to schizophrenia**

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The ancestral susceptibility hypothesis has been proposed to explain the existence of common susceptibility alleles. Some ancestral alleles, reflecting ancient adaptations, may be poorly adapted to the more contemporary environmental conditions giving rise to an increased risk to suffer some common disorders. In order to test this hypothesis in schizophrenia, we focus on monoamine oxidase B (MAOB). This gene is involved in deamination of several monoamines, including both xenobiotic amines present in several foods, as well as neurotransmitters such as dopamine. In addition, preliminary analysis based on phase I HapMap data suggested that recent natural selection have acted on this locus. We further explored the existence of this recent positive selection using a test based on extension of linkage disequilibrium (LD) to large distance at the specific selected haplotype taking data from HapMap phase II, and searched for association of the ancestral haplotypes to schizophrenia in a sample of 532 schizophrenic patients and 597 controls from Spain. Our analysis suggests the existence of a haplotype of MAOB subject to recent selection. In agreement with the ancestral susceptibility hypothesis, the ancestral haplotypes were significantly over-represented in patients ( $P = 0.047$ ). These haplotypes confer an increased risk to schizophrenia, restricted to males ( $P = 0.024$ , OR = 1.41, 95% CI 1.01-1.90). Thus, pending on replication studies, MAOB seems to fit the ancestral susceptibility model, opening a new strategy to search for common schizophrenia susceptibility genes by focusing in those functional candidate genes subject to recent positive selection.

**P06.264****Support for the involvement of NRG1 in schizophrenia in Iranian population: Evidence from a case: control association study**

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It has been accepted widely that schizophrenia is a multifactorial disorder and evidence in support of the involvement of genetic factor in pathology of schizophrenia is compelling. As a result of extensive study, a list of genes has emerged as candidate genes for schizophrenia. Research into genetics of schizophrenia has found Neurgulin1 (NRG1) among the most promising candidate genes for schizophrenia. For the first time in 2002, Steafansson et al suggested NRG1 as a candidate susceptibility gene for schizophrenia in a linkage study carried out in an Icelandic population. Since then, the association of NRG1 with schizophrenia was further confirmed by several studies in different population. However, analyzing allele and haplotype frequencies of NRG1 in distinct populations have yielded varying results and also different alleles or haplotypes have been associated with schizophrenia. Meanwhile, some studies have failed to replicate the association. In this work we attempted to study the association of two NRG1 polymorphisms with schizophrenia in Iranian population. SNP8NRG241930 and SNP8NRG221533 were studied in 100 cases of schizophrenia matched with 100 healthy individuals. For the first time in Iranian population, we showed a positive association between NRG1 and Schizophrenia. Our statistical analyses indicate that there are significant differences in allelic and genotypic frequencies between two studied groups.

**P06.265****Autosomal recessive form is the predominant pattern in Iranian patients with Severe Combined Immune Deficiency Disease**

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Severe Combined Immune Deficiency is a X-linked and Autosomal Recessive disorder. World investigations showed nearly 48% X-linked inheritance which caused by mutations of the IL-2RG. This form of SCID is characteristically T-/NK-/B+. Also many genes involved in Autosomal Recessive pattern. The aim of this study was to determine the inherited pattern of SCID affected families in Iranian patients.

**Methods:** Genomic DNA of 5 male unrelated patients with T-B+ clinical symptoms of SCID was purified from peripheral blood. The sequencing of IL2RG was performed to find out if they harbour any mutation in these exones or not.

**Result:** We analyzed the IL-2RG (The X-linked mode of inheritance) of the patient for mutations by direct genomic sequencing, but found it to be normal, suggesting an autosomal mode of inheritance of SCID.

**Discussion:** About 48% of SCID patient were reported to have IL2RG deficiency in the word but our result shows that autosomal recessive pattern is the predominant in Iranian SCID patients even the number of patients is not enough to say strongly that. Because of consanguinity marriage it is also very hard to say that the patient who lost boy because of SCID had cause from IL2RG gene. Because of complexity of SCID we suggest that for those family who lost boy first linkage with Chromosome X and then if it is negative for that linkage with other causative genes. This is pilot study and much more investigation need to clarify that which gene is more frequently causative in Iranian Patient.

**P06.266****Association of a GABA(B) gene haplotype and Temporal Lobe Epilepsy**

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There is experimental evidence that dysfunctions of the GABA(B) receptor I gene (GABBR1) are implicated in the pathogenesis of temporal

lobe epilepsy (TLE). Using a candidate gene approach, we recently illustrated that the G1465A polymorphism in the GABBR1 is a strong risk factor for non-lesional TLE. Afterwards, other studies observed no evidence of association. To better investigate this association, we set out to examine whether common variations in GABBR1 predisposes to the development of non-lesional TLE. We performed a genetic association analysis of GABBR1 sequence variants by evaluating three SNPs and an (AC)n repeat in a sample of 236 patients with non-lesional TLE and 380 age and geographic origin matched healthy controls. The program UNPHASED was used to compare genotype, allele and haplotype frequencies between cases and controls, including age and gender as covariates in the model.

We found that each variation conferred an increased risk of disease development. In addition, the four-SNP haplotypes were associated with the disease.

Our data show that genetic variability in GABBR1 is highly associated with susceptibility to non-lesional TLE, further corroborating our earlier observations. Further studies in different TLE patient-control cohorts are needed.

**P06.267****Interleukin-23 receptor (IL23R) gene polymorphisms in patients with Sjögren syndrome**

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<sup>1</sup>Department of Medical Genetics and Child Development, University of Pécs, Pécs, Hungary, <sup>2</sup>Medical and Health Science Center, Institute for Internal Medicine, 3rd Department of Medicine, University of Debrecen, Debrecen, Hungary. Sjögren syndrome (SS) is an autoimmune disease that mainly effects the exocrine glands. It is clinically characterized by keratoconjunctivitis sicca and xerostomia. Duerr et al. (Science, 2006; 314:1461-1463) found an association between Crohn's disease and the interleukin-23 receptor gene using genome-wide association; amongst the reported SNPs the 3'-UTR C2370A (rs10889677) conferred risk for Crohn's disease, the Arg381Gln (rs11209026) showed the strongest protective effect and the Pro310Leu (rs7530511) had no association with the disease. Since the IL23 pathway is known to associate also with other autoimmune diseases, we theorized that these SNPs might also have significance in the development of SS. We performed genotyping using DNA samples collected from 156 patients with SS and 182 unrelated, healthy controls. The genotypes were analysed using PCR/RFLP-methods. We found no significant difference between the allele frequencies of the two groups, as for rs10889677 the distribution of genotypes were CC 45.5%, CA 43.6%, AA 10.9% in patients with SS, while we observed CC 45.6%, CA 48.4%, AA 6.04% in the control group (not significant). For rs11209026 the genotypes were GG 87.8%, GA 12.2% and GG 86.3%, GA 13.7% in the examined groups. Similar results were obtained for rs7530511 also: CC 73.7%, CT 22.4%, TT 3.85% and CC 72.5%, CT 25.3%, TT 2.20% in the groups of patients and controls, respectively. Our results suggest that these SNPs do not play a role in the development of Sjögren syndrome.

**P06.268****The STK11-PRKAA2-CRTC2 genes are play a major role in the genetic susceptibility to type 2 diabetes**

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The activation of hepatic LKB1-AMPK-TORC2 signaling pathway, which is the probable target for the antidiabetic drug metformin, have a key role for development of type 2 diabetes (T2D). 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 *STK11* and *CRTC2* by direct sequencing 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 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 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 type 2 diabetes. In conclusion, this study has found a weak evidence that *STK11*, *PRKAA2*, or *CRTC2* polymorphisms contribute to the susceptibility to T2D in Japanese.

#### P06.269

##### Extremely High Carrier Frequency of SMA in Iranian Population

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Spinal muscular atrophy (SMA) is one of the most common autosomal recessive diseases, affecting approximately one in 6000 to 10000 live births and with a carrier frequency of approximately one in 40 to 60. About 94% of individuals with clinically typical SMA lack both copies of SMN1 exon 7.

Carrier frequency studies of SMA have been reported to be variable in different population and no population-based studies has been done in Iran, however our observations indicate that the incidence of SMA is much higher in Iranian population partly because of high rate of consanguineous marriages. The copy number of SMN1 gene was determined in 400 normal individuals by quantitative real - time PCR with SYBR Green I dye. The comparative threshold cycle (Ct) of each sample was calculated and albumin was used as a reference gene. The homozygous SMN1 deletion  $\Delta\Delta Ct$  ratio of patient was 0.00 and the hemizygous SMN1 deletion  $\Delta\Delta Ct$  ratio of obligate carriers ranged from 0.29 to 0.55. The  $\Delta\Delta Ct$  ratio of 92 persons among 400 normal individuals was within the carrier range, 0.31-0.57.

Our data indicated that the carrier frequency of SMA in Iranian population is higher (1 out 5) than other countries.

#### P06.270

##### Determination of the SMN1 and SMN2 copy number based on real-time PCR in Hungarian SMA families

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Spinal muscular atrophy (SMA) is characterized by progressive muscle weakness caused by degeneration of the spinal anterior horn cells. Patients with SMA have been classified into three types on the basis of clinical severity. The survival motor neuron gene is present in two copies, SMN1 and SMN2, which differ by only five nucleotides. Only SMN1 gene provides fully functional protein due to exon 7 skipping in SMN2. On the other hand, the SMA phenotype can be modified by the presence of several copies of SMN2.

SMA is a common and fatal disorder, therefore the carrier detection is essential for prevention and proper genetic counseling. Therefore, estimation of the SMN1 and SMN2 copy number in patients by real-time PCR has been recently introduced in Hungary. This technique is used also for the detection of possible compound heterozygotes.

Until now, SMN1 copies were determined in 25 affected patients and their 132 family members and twenty controls. Seven patients were identified as being compound heterozygotes and thus, the diagnosis of SMA was assumed. The intragenic pointmutations will be identified later on by sequencing. Additionally, 151 SMA patients with undefined genetic diagnosis still have to be tested for the SMN1 copy number. For urgent family planning, 24 relatives of the known carrier parents were tested and 8 were confirmed as carriers of the common SMN1 mutation. Additionally, SMN2 copy number were estimated in 64 patients and in 33 family members and a good correlation was found between copy number and severity of the disease.

#### P06.271

##### Highly significant association between Contactin Associated Protein-like 2 (CNTNAP2) and non-word repetition in a language impaired sample

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Specific Language Impairment (SLI) is defined as a substantial delay in the development of language despite normal development in other areas and in the absence of accompanying neurological conditions like autism. Despite the diagnostic division between autism and SLI, the two disorders share many clinical features and researchers have proposed that they may share risk factors and involve mutual neurodevelopmental pathways. The lack of any clear genetic candidates has precluded the validation of this hypothesis at the molecular level. However, converging evidence from genetic research has recently implicated members of the neurexin gene family in autism. In the present study we therefore investigated a neurexin gene, Contactin Associated Protein-like 2 (CNTNAP2), within families affected by SLI. We typed 37 SNPs within 184 families ascertained by the SLI Consortium (SLIC). Quantitative TDT (QTDT) was used to assess marker-trait association for three language-related measures. Expressive and receptive language abilities were assessed with the Clinical Evaluation of Language Fundamentals (CELF-R) and a test of non-word repetition (NWR) was used to measure phonological short-term memory. We found a highly significant level of association (max  $P=0.00005$ ) between NWR and a cluster of 9 SNPs within the CNTNAP2 gene. A suggestive level of association was also observed in this region to both CELF measures (max  $P=0.003$ ). These results were supported by subsequent haplotype and regression analyses. Our findings support the existence of shared genetic risk factors between SLI and autism, a conclusion which yields important consequences for the conceptualisation, diagnosis and treatment of these disorders.

#### P06.272

##### An investigation of dyslexia risk loci in families with Specific Language Impairment, (SLI), implicates *KIAA0319* as a shared genetic factor

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Specific Language Impairment, (SLI), is defined as a considerable developmental delay in the acquisition and/or use of language in the absence of other diagnostic features such as hearing loss and autism. There is substantial co-morbidity of SLI with developmental dyslexia, in which children are described as having unexpected difficulties in learning to read, write and spell. This has lead researchers to investigate the possibility of shared risk factors for the two disorders. Several genomic regions have shown consistent association to dyslexia on chromosomes 2, 6 and 15. We typed 16 SNPs identified within these regions in 175 language-impaired families, ascertained by the SLI Consortium (SLIC). Quantitative TDT (QTDT) was used to assess marker-trait association using both orthogonal (within family) and total (within- and between- family) association to three reading-related measures. These measures, taken from the Wechsler Objective Reading Dimensions (WORD), assess basic reading, spelling, and reading comprehension. Single SNP orthogonal analysis identified association of these measures to SNPs within *MRPL19/C2ORF3* on chromosome 2, and *DCDC2* on chromosome 6. Total association was found to the same SNP in *MRPL19/C2ORF3*, and also to a SNP within *KIAA0319* on chromosome 6. We used the SNP data to reconstruct haplotypes that had previously been reported to be associated with dyslexia. In this analysis, association was replicated only in the *KIAA0319* gene and with all reading-related measures. This investigation therefore indicates that the loci identified by studies of dyslexia, and in particular *KIAA0319*, may also contribute to reading ability in language impaired populations.

**P06.273****Intronic sequence changes may have unpredictable effects on splicing****F. Joncourt, S. Gallati;***Human Genetics, Department of Pediatrics, University Hospital, Berne, Switzerland.*

Mutation scanning often identifies yet undescribed sequence changes in genomic DNA, which are not easily classified as either pathogenic or neutral solely by sequence inspection. Increasing knowledge about the splicing process has revealed its great complexity: In addition to the well known splice-site consensus sequences in recent years a multitude of regulatory elements have been identified both within introns as well as within exons. In order to assess / confirm their pathogenic nature, we have analysed the effect on splicing of several yet functionally uncharacterized intronic sequence changes in different genes by analysing their respective RNA's. Lymphocytes from patients were immortalized, RNA was extracted and reverse transcribed. The cDNA was then amplified by PCR and analysed by PAGE or agarose gel electrophoresis. Bands with altered electrophoretic mobility were isolated, purified and sequenced. Two mutations located within the conserved donor and acceptor splice sites respectively (NF1: c.3496+1G>A and SPG4: c.1688-2A>G) were shown to lead to exon skipping as expected. c.357+2T>A identified in a patient's DMD-gene, however, resulted in the inclusion of 31 bases of intronic sequence into the mRNA leading to a frameshift, while exon skipping would have left the reading frame intact. This latter case further illustrates that the effect of an unknown mutation on the splicing process can not easily be predicted. The results emphasize the need for functional characterization of newly described sequence changes.

**P06.274****SPRY1 molecular analysis in subjects with ureteral duplicity****L. Artifoni<sup>1</sup>, E. Benetti<sup>2</sup>, S. Negrisolo<sup>1</sup>, S. Centi<sup>1</sup>, G. Caridi<sup>3</sup>, G. Ghiggeri<sup>3</sup>, L. Murer<sup>1,2</sup>;**

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Studies on murine models demonstrated that Sprouty1 protein, encoded by Spry1 gene, modulates Gdnf/Ret signal, which activates a crucial gene network in urinary tract development. Spry1-knockout mice develop supernumerary ureteric buds, that result in multiple ureters and kidneys. In the literature, there only one report about mutational analyses of human SPRY1 gene, the homologue of murine Spry1, even if the gene is known to be expressed in fetal renal tissue.

We carried out mutational analysis of SPRY1 gene in patients with ureteral duplicity: 23 isolated and 4 familial cases. On each DNA sample, the coding region and 5'UTR were analysed by SSCP and all PCR products were then directly sequenced (ABI PRISM 3100 Applied Biosystem). DNA from 6 subjects without kidney and urinary tract anomalies was used as control group.

We detected 5 polymorphisms (SNPs), previously reported in databases, and 1 nucleotide substitution, which has never been reported. The frequency of this substitution was estimated in 127 umbilical cord blood DNA samples and was 0.094. In order to understand SPRY1 role in renal development, mutational analysis will be extended to a population of subjects with different malformative nephrouropathies and association studies with the detected polymorphisms will be performed.

**P06.275****Transcriptomic analysis of statin treated rat skeletal muscle cells****M. J. Ko, H. S. Choi, H. S. Jeong, J. I. Ahn, S. Y. Kim, H. J. Chung;***National Institute of Toxicological Research of Korea, Seoul, Republic of Korea.*

Statins are competitive hydroxy-3-methyl glutaryl coenzyme A(HMG-CoA) reductase inhibitors that inhibit the synthesis of cholesterol from mevalonic acid. Statins are the drugs most frequently used to reduce plasma cholesterol levels and decrease cardiovascular events. However, the side effects associated with the use of statins have been highlighted by the withdrawal of cerivastatin from the market in 2001, but little is currently known about the effect of statins on the RNA expression profile of skeletal muscle cells and mechanism of myopathy.

To address this issue, we used rat L6 myoblast cells, which can differentiate into myocytes, in this study. We treated cells with cerivastatin which had developed potent myopathy or pravastatin relatively less toxic than cerivastatin, and measured cell viability using MTT assay. MTT assay showed significant concentration-dependent decrease of cell viability by treatment of statins and revealed cerivastatin is much more toxic than pravastatin. It seems that DNA microarrays could be very helpful not only to predict drug-induced toxicity, but also to better understand the mechanism of actions of drug. Using DNA microarrays, we discovered 522 genes that are specifically responsible to cerivastatin-induced muscle cell toxicity and these genes correctly classified as muscle toxicity group. Moreover fifteen genes that are the potential candidates as myopathy biomarkers were selected. Among them, seven genes are the sensitive genes that were showed response even at the low toxic dose, and eight genes are responded in dose-dependent manner.

**P06.276****Genetic association study of Kalirin and Ropporin with ischemic stroke****T. Krug<sup>1</sup>, H. Manso<sup>1,2</sup>, L. Gouveia<sup>3</sup>, B. V. Fonseca<sup>1</sup>, I. Albergaria<sup>2</sup>, G. Gaspar<sup>2</sup>, M. Correia<sup>4</sup>, M. Viana-Baptista<sup>4</sup>, R. M. Simões<sup>5</sup>, A. N. Pinto<sup>6</sup>, R. Taipa<sup>4</sup>, C. Ferreira<sup>7</sup>, J. R. Fontes<sup>7</sup>, M. R. Silva<sup>8</sup>, J. P. Gabrie<sup>8</sup>, I. Matos<sup>9</sup>, G. Lopes<sup>4</sup>, J. M. Ferro<sup>3</sup>, A. M. Vicente<sup>1,2</sup>, S. A. Oliveira<sup>1</sup>;**

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Introduction: Cerebrovascular and cardiovascular diseases such as stroke and coronary artery disease (CAD) are the leading causes of death and disability worldwide. They are complex disorders resulting from the interplay of genetics and environment, and may share several susceptibility genes. Recently, an association-mapping study in the chromosome 3 linkage peak for CAD found that the rs9289231 SNP in the Kalirin gene (*KALRN*) was associated with CAD in multiple datasets. *KALRN* is involved, among others, in the inhibition of inducible nitric oxide synthase, in guanine exchange factor activity, and in the Rho GTPase-signaling pathway. The goal of the present study was to determine whether SNPs or haplotypes in the *KALRN* gene region, which includes the Ropporin gene (*ROPN1*), predispose to ischemic stroke (IS) in a cohort of Portuguese patients and controls.

Methods & Materials: We genotyped 27 tagging SNPs in the *KALRN-ROPN1* chromosomal region, on 565 IS patients and 518 unrelated controls, and performed single-marker association tests.

Results: Intronic SNP rs11712619 in *KALRN* was associated with IS risk in unadjusted (allelic and genotypic p=0.003) and adjusted (log-additive model p=0.027, adjusted for age-at-examination, hypertension, diabetes, ever smoking/drinking) tests. A block of six SNPs (from rs2280422 to rs12637456), located mostly in the *ROPN1-KALRN* intergenic region, had a modest (0.02< p <0.05) allelic and genotypic association with IS risk when unadjusted for co-variates. The rs9289231 SNP was not associated with IS in any test performed.

Conclusions: This study suggests that variants in the *ROPN1-KALRN* region may constitute risk factors for stroke in the Portuguese population.

**P06.277****Polymorphisms in PDE4D gene region are associated with ischemic stroke recovery****H. Manso<sup>1,2</sup>, T. Krug<sup>1</sup>, T. Magalhães<sup>1,2</sup>, B. V. Fonseca<sup>1</sup>, J. Sobral<sup>1,2</sup>, I. Albergaria<sup>2</sup>, G. Gaspar<sup>2</sup>, J. M. Ferro<sup>3</sup>, S. A. Oliveira<sup>1</sup>, A. M. Vicente<sup>1,2</sup>, t. PORTUGuese Stroke GENetics (PORTSGEN) Group<sup>4</sup>;**

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Stroke is a major cause of permanent disability in developed countries. It is therefore of major importance to understand how to reduce brain damage and how to improve patient's recovery. Evidence from animal studies and an association between family history of stroke and stroke outcome suggests the existence of genetic factors influencing stroke recovery. The PDE4D gene, a regulator of cAMP degradation,

is a possible candidate because cAMP levels in immunocompetent cells influence the release of inflammatory mediators and relevant cytokines, which are important mediators of stroke recovery. Forty five SNPs covering genetic variation in the 5' region of the PDE4D gene, including SNPs previously associated with stroke risk in the Icelandic population, were genotyped in 430 ischemic stroke patients and tested for association with functional recovery. Stroke recovery was assessed 3 months after a stroke episode, using the modified Rankin Scale (mRS). Patients were classified in two groups: good (mRS<=1) or poor recovery (2<=mRS<6). Binary logistic regression analysis was carried out to adjust for clinical severity parameters and risk factors with a significant impact on stroke recovery. Seven SNPs were associated with mRS ( $0.00098 < P < 0.048$ ) after adjusting for significant co-variates - history of hypertension, aphasia, paresis, consciousness disturbance and medical complications during hospitalization. Five haplotypes, including at least one of the associated individual SNPs, were also associated with stroke outcome ( $0.0056 < P < 0.0367$ ). These results suggest that PDE4D gene variants may contribute to individual variability in ischemic stroke recovery, possibly by playing a role in the neuroinflammatory processes that occur after stroke.

#### P06.278

#### Association analysis of 677C>T polymorphic marker of MTHFR gene and suicide

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Suicide is almost always a complication of a psychiatric illness, the most common of which is a mood disorder that generally accounts for 60% of cases. It has been reported that a functional single gene polymorphisms 677C>T (rs1801133) of methylenetetrahydrofolate reductase gene (MTHFR) is involved in pathogenesis of depression, and also of schizophrenia and bipolar disorder. The aim of our study was to conduct the association analysis of 677C>T polymorphism of MTHFR gene and suicidal behavior in 310 patients (134- Russians, 107- Tatars) and 434 health volunteers (169- Russians, 248- Tatars) from Bashkortostan (Russia) using PCR-RFLP technique. The distribution of genotype frequencies was in accordance with Hardy-Weinberg equilibrium. No significant differences in either allele or genotype frequencies of MTHFR 677C>T polymorphism were found between suicidal and control groups in Russian population. However in Tatar population MTHFR\*T allele ( $X_2 = 16.48$ ;  $p < 0.001$ ;  $df = 1$ ;  $OR = 2.13$ ; 95%CI 1.47-3.08) and MTHFR\*C/T genotype ( $X_2 = 21.14$ ;  $p < 0.001$ ;  $df = 1$ ;  $OR = 2.95$ ; 95%CI 1.85-4.737) were shown to be significantly associated with suicidal behavior. Moreover, MTHFR\*C allele ( $X_2 = 16.48$ ;  $p < 0.001$ ;  $df = 1$ ;  $OR = 0.47$ ; 95%CI 0.33-0.68) and MTHFR\*C/C genotype ( $X_2 = 21.95$ ;  $p < 0.001$ ;  $df = 1$ ;  $OR = 0.33$ ; 95%CI 0.21-0.53) are reported to be protective markers of suicidal behavior in Tatar population. Our study reports the involvement of MTHFR gene in predisposition to suicidal behavior; and it is shown that the effect is influenced by ethnicity.

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#### P06.279

#### Association of a CD24 gene polymorphism with susceptibility to systemic lupus erythematosus

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The aim of this study was to determine the potential role of the CD24 A57V gene polymorphism with SLE. Our study includes three Caucasian cohorts: cohort 1 from Spain (696 SLE patients and 539 controls),

cohort 2 from Germany (257 SLE patients and 317 controls) and cohort 3 from Sweden (310 SLE patients and 247 controls). The CD24 A57V polymorphism was genotyped using a PCR system with pre-developed TaqMan allelic discrimination assay. In the Spanish cohort we found a statistically significant difference in the distribution of the CD24V allele between SLE patients and controls ( $P < 0.0001$ ,  $OR = 3.6$ , 95%CI 2.13-6.16). In addition, the CD24V/V genotype was increased in SLE patients ( $P < 0.00001$ ,  $OR = 3.7$ , 95%CI 2.16-6.34) compared with controls. Additionally, we sought to replicate this association with SLE in a Swedish and a German SLE population. A similar trend was found in the German set, where the CD24V/V genotype and the CD24V allele were more frequent in SLE patients than in controls, although this difference did not reach statistical significance. No differences were observed in the Swedish set. A meta-analysis of the Spanish and German cohorts demonstrated that the CD24V allele has a risk effect in SLE patients (Pooled  $OR = 1.25$ , 95%CI 1.08-1.46,  $P = 0.003$ ). In addition, homozygosity for the CD24V risk allele significantly increased the effect (Pooled  $OR = 2.19$ , 95%CI 1.50-3.22,  $P = 0.00007$ ). Our data suggest that the CD24A57V polymorphism plays a role in the susceptibility to SLE in a Spanish population.

#### P06.280

#### Genetic polymorphism of Eta-1/Osteopontin is associated with systemic lupus erythematosus in Korea

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**Background:** The aim of this study is to investigate the association with this SNP at position nucleotide 9250 (C>T) in OPN gene and susceptibility to SLE patients firstly. Also, we try to compare the allele frequency of Korean with them of USA and Japanese.

**Materials and Methods:** A total of 40 Systemic lupus erythematosus (SLE) patients and 104 healthy controls were studied. SNP located at position 9520 in the Osteopontin (OPN) gene were genotyped using the restriction fragment length polymorphism (RFLP). The wild-type sequence contains a C while the polymorphism variant is a T (C>T), which results in the appearance of an Alu I restriction site.

**Results:** The gene frequencies of C/C, C/T, and T/T at position 9250 on the Eta-1/osteopontin gene in SLE patients were 37.5%, 12.5%, and 50.0%, respectively, compared with 6.7%, 27.9%, and 65.8% in controls ( $p < 0.05$ ). The allele frequencies of C and T at this position in such SLE patients were 43.75 and 56.25, whereas those in controls were 20.7 and 79.3 ( $p < 0.05$ ), respectively. The allele frequencies found in the present study were compared with those coding SNP described in the USA database; 60 and 39 (USA) vs 20.7 and 79.3 (Korea) ( $p < 0.05$ ).

**Conclusions:** Those findings suggest Eta-1/osteopontin genetic polymorphism and allele frequencies were significantly association with SLE patients. Also we observed the difference of allele frequencies in our controls and in USA controls, these differences may be caused by a difference in incidence of SLE patients between the two countries.

#### P06.281

#### Analysis of the genetic variability in the TFF gene cluster

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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. Genetic variation in these genes could influence inflammation progression to gastric cancer. To study association between polymorphisms in the *TFF* genes and gastric carcinogenesis, the purpose of this study was to characterize variability in the *TFF* cluster.

We selected *TFF* polymorphisms from public databases and used RFLP and SSCP/HD analysis to validate them in a control sample group. SSCP/HD was also used to scan the *TFF* genes for novel variants. Validated polymorphisms were genotyped in a larger population by real-time PCR and, one of them, by the recently developed *High*

*Resolution Melting* technology in the LightCycler 480. We performed linkage disequilibrium (LD) and haplotype analysis using the Haploview and SNPstats programs.

Six of the nine polymorphisms initially selected from the public databases were validated in our population. SSCP/HD analysis allowed for the identification of 9 novel SNPs (3 in *TFF1*, 4 in *TFF2* and 2 in *TFF3*), 7 of which could be functional, and a novel insertion polymorphism c.\*66\_67InsCTT, in the 3'UTR region of *TFF2*. Preliminary LD analysis indicates that each *TFF* gene is located in independent LD blocks and that high LD exists between SNPs in *TFF2*. Genotyping of these polymorphisms in case-control studies of gastric cancer is at present underway.

#### P06.282

#### Investigation of genetic component of susceptibility to atopic bronchial asthma and tuberculosis: xenobiotic-metabolising enzymes.

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The relationships of polymorphic variants of the genes encoding xenobiotic-metabolising enzymes (*CYP2C19*, *CYP2E1*, *GSTM1*, *GSTP1*) with atopic bronchial asthma and lung tuberculosis, were studied in people of Tomsk region. An association of a deletion polymorphism of the *GSTM1* gene and a 7632T>A exchange of the *CYP2E1* gene with bronchial asthma were revealed ( $p=0.008$  and  $p=0.049$ , respectively). Prevalence of a "null" genotype of the *GSTT1* gene was significantly different in groups of patients with various severity of bronchial asthma ( $p=0.045$ ). It was shown that 313G/G genotype of the *GSTP1* gene is a factor of resistance to tuberculosis (OR=0.43; 95%CI: 0.20-0.91;  $p=0.026$ ). 681G>A polymorphism of the *CYP2C19* gene was associated with scope of a pulmonary tissue damage ( $p=0.040$ ) and with amount of erythrocytes in tuberculosis patients ( $p=0.027$ ). 313A>G polymorphism of the *GSTP1* gene was associated with variability of alanine aminotransferase levels ( $p=0.021$ ). The combination of the *GSTM1* +/+ and *GSTP1* 313G/G, played a protective role for the both studied diseases (OR=0.10;  $p=0.018$  for the asthma; OR=0.37;  $p=0.045$  for the tuberculosis). This suggests that the genotypes and their combinations of *CYP2C19*, *GSTT1*, *GSTM1* and *GSTP1* genes have influence on predisposition and clinical polymorphisms of atopic asthma and lung tuberculosis.

#### P06.283

#### Sex-specific genetic determinants in plasma levels of fibrinogen

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A major challenge of biomedical research is the identification of risk factors for complex diseases. One well-established risk factor for cardiovascular disease is the plasma fibrinogen levels. These levels are influenced largely by genetic factors. But the exact nature of these genetic factors is unknown.

Our aim was to localize QTL responsible for the plasma levels of fibrinogen. We present the results of a Genome Wide Scan for fibrinogen levels that includes a gender-specific analysis in 21 Spanish families from the GAIT (Genetic Analysis of Idiopathic Thrombophilia) Project. We used 500 DNA microsatellites scattered throughout the genome. Our results revealed a highly significant LOD score (3.52, nominal  $p=0.00003$ ) on Chromosome 17 that was only detected in females, indicating that a QTL on this chromosome was responsible for fibrinogen levels only in women.

A bioinformatic study in the region of Chromosome 17 revealed the presence of some hormone-related genes (steroid hormone receptors) that affect fibrinogen levels and that could explain this gender-specific finding.

To our knowledge, this is the first report of a gender-specific QTL related to cardiovascular disease. It demonstrates that a gender-specific

genetic analysis can increase our ability to detect phenotypes that are affected by sex. We hope that our results will help to understand the regulation of fibrinogen plasma levels that determine the susceptibility of cardiovascular disease. Our results may provide a template for future genetic studies of quantitative traits affecting complex diseases.

#### P06.284

#### Genetic variants of the TBX15 gene associated with thyroid cancer susceptibility

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Association studies to identify susceptibility genetic factors for thyroid cancer have been appeared recently. Our initial case-control association studies had shown that the rs2145418 and the rs4658973 markers that map 377 kb apart on the 1p12 region have an independent association with thyroid cancer susceptibility. Next we have expanded these initial studies by genotype and haplotype analysis in the region containing the two markers. Here we present the results related to the rs2145418 region. Thus, the MassArray technique was used to genotype 136 control individuals and 201 thyroid patients. First we have confirmed our previous results, by genotyping the rs2145418 SNP along with two SNPs surrounding this marker. Only the rs2145418 polymorphism showed association with thyroid cancer. Consequently, our hypothesis that rs2145418 could reside in a regulatory sequence of casual genes mapping near or at certain distance of this marker can not be discarded. In addition, we have studied nine SNPs that cover the TBX15 gene sequence, this gene maps 550 kb downstream of rs2145418. Four of these SNPs lying on the 5'-UTR region have shown significant association with thyroid cancer ( $p<0.05$ ). Furthermore, the association of each SNP alone is less significant than that of a variant haplotype (TACT) of the four 5'-UTR markers (OR, 2.18; 95% CI, 1.23-3.88;  $P=0.0085$ ). The TBX15 gene is involved in developmental processes; thus, based in our results, we can conclude that the TBX15 gene may be involved in thyroid cancer risk.

#### P06.285

#### Polymorphisms of TLR2, TLR4 and CD14 genes and risk of meningococcal and pneumococcal infections in children

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The Toll-like receptors (TLRs) and CD14 are factors of the innate immune defences to microbial infections and seem to play an important role in signalling of molecules of pathogens and of endogenous proteins related to immune activation and influence the susceptibility to and the evolution of several diseases as can be sepsis, asthma and atherosclerosis.

In the present study, we have genotyped the polymorphisms p.R677W and p.R753Q within TLR2 gene, p.D299G of TLR4 gene and c.-159C>T of CD14 gene in 157 blood samples from children admitted in the Critical Care Unit of the Hospital Niño Jesús (Madrid) with meningococcal (Gram-negative) ( $n=55$ ) or pneumococcal (Gram-positive) ( $n=102$ ) infections. Sixty six controls were genotyped in order to compare the genotypic frequencies.

Genotypic frequencies of TLR2 polymorphisms were clearly different in both groups of patients, when compared to control population, especially for the p.R753Q polymorphism, being more frequent the p.753Q allele ( $p=0.0004$  in meningococcal infections, and  $p=0.0005$  in pneumococcal infections).

Conversely, TLR4 polymorphism did not show different genotypic distribution when compared to control population. Finally, the study of the polymorphism of the CD14 showed different distribution when compared to controls, with a  $p=0.0113$  in meningococcal infections and  $p=0.0353$  in pneumococcal infections.

No differences were found among both groups of studied patients.

These results confirm the key role of the innate immunity system in predisposition to severe infections. For both studied bacteria, the main risk factor is the p.753Q allele followed by the CD14 promoter polymorphism. TLR4 polymorphism did not influence the risk of suffering infections.

**P06.286****Novel assay for TLR4 single nucleotide polymorphisms testing in patients with acute coronary syndrome**

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Our aim was to investigate occurrence of: A896G(Asp299Gly), C1196T(Thr399Ile) and other single nucleotide polymorphisms (SNP) of toll-like receptor TLR4 in patients with ACS and healthy controls. TLR4 is activated in the innate immunity response to lipopolysaccharide LPS. TLR4-dependent reaction is involved in the pathogenesis of atherosclerosis i.e. acute coronary syndrome (ACS). SNP A896G and SNP C1196T of TLR4 are postulated to occur more often in patients with ACS.

Blood samples were collected from 50 patients with ACS within 24 hours from admission to ICCU and from 100 healthy controls. Genomic DNA was extracted and TLR4 genetic variations A896G and C1196T have been analyzed by genotyping with LightTyper system. Unknown SNP's of TLR4 were investigated with MSSCP.

Frequency of A896G(Asp299Gly) and C1196T(Thr399Ile) allele were in ACS patients and controls: G - 0,03 and 0,01, resp., A - 0,97 and 0,99, resp.; C - 0,955 and 0,95, resp. and T - 0,045 and 0,05, resp. Very rare homozygous G/G variant of A896G was found in one patient with ACS. New cost-effective genotyping technique has been developed - allele specific PCR reaction was performed with primer length modification with poli(T), which allows analysis of multiplex PCR reaction.

Frequency of A896G and C1196T allele did not differ significantly among patients with ACS and controls. However, relatively small number of patients requires caution in extrapolating results to population study. The second part of our study will be quantified assessment of TLR4 expression: in controls, in patients during ACS and then in stable period of CAD.

**P06.287****Relation of *TPH1* gene polymorphic variants to personality traits associated with psychiatric disorders**

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Individual differences in personality are influenced by both environmental and genetic factors. Enormous studies indicated association between *TPH1* A218C polymorphism and personality disorders (characterized by impulsivity, anxiety and low extraversion). It is likely that genetically mediated variability of the *TPH1* gene functioning can contribute to individual differences in personality traits.

We aimed to define genotype effect of *TPH1* A218C polymorphism and to check possible *TPH1*\*gender and *TPH1*\*ethnicity interaction effect on personality traits (assessed with the EPI and TCI questionnaires). We recruited 602 healthy individuals (men-206, women-396) of Caucasian origin (Russians-214, Tatars-388) from Russia (mean age±SD, 19.85±2.43 years).

Association between C-allele and higher Extraversion ( $p=0.001; F=11.127$ ), and lower Harm Avoidance ( $p=0.027; F=4.922$ ) was observed, that was also reported in women and Tatars. Gender and ethnicity specific differences on personality were detected, with women and Tatars scoring higher on Harm Avoidance, Tatars scoring lower on Extraversion. MANOVA (carried out with gender and ethnicity as second factors) revealed the influence of *TPH1*\*ethnicity interaction on Extraversion ( $p=0.032; F=4.634$ ) observed due to the differences in this trait between Tatars with C-allele and A/A-genotype ( $p=0.000; F=15.779$ ); Tatars with A/A-genotype and Russians with C-allele ( $p=0.000; F=19.770$ ); Tatars and Russians with A/A-genotype ( $p=0.007; F=7.820$ ). The same pattern of *TPH1*\*ethnicity interaction influence on Extraversion was demonstrated in women ( $p=0.043; F=4.139$ ).

Current findings suggest that the variance in Extraversion and Harm Avoidance could be explained by *TPH1*\*ethnicity interaction, although the single *TPH1* genotype and ethnicity effect occurs.

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**P06.288****TUB gene polymorphisms are associated with anthropometry and eating behavior in middle-aged women**

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The *TUB* gene, encoding an evolutionary conserved protein, is highly expressed in the hypothalamus and might act as a transcription factor. Mutations in *TUB* cause late-onset obesity, insulin-resistance and neurosensory deficits in mice. An association of common variants in the *TUB* gene with body weight in humans has been reported. The aim was to investigate the relationship of single nucleotide polymorphisms (SNPs) of the *TUB* gene (rs2272382, rs2272383 and rs1528133) with both anthropometry and macronutrient intake in general population. The association of SNPs in *TUB* with body composition and macronutrient intake from a validated food frequency questionnaire was studied in a population-based study of 1680 middle-aged Dutch women.

The minor allele C of the rs1528133 was significantly associated with increased weight (+1.88 kg,  $P = 0.022$ ) and BMI (+0.56 units,  $P = 0.05$ ). Both AG and AA genotypes for the rs2272382 were associated with an increased energy intake from carbohydrates (0.69%,  $P = 0.04$  and 1.68%,  $P = 0.003$ , respectively), mainly because of a higher intake of mono- and disaccharides. Both these SNPs were also associated with a higher glycemic load (GL) in the diet. The GL was higher among those with AG and AA genotypes for the variant rs2272382 than among the wild types (+1.49 and +3.89 units, respectively). Carriers of the minor allele C of rs1528133 were associated with an increased GL of 1.85 units compared with non-carriers.

Our results suggest that the effect of *TUB* on body composition might be due to the increased intake of carbohydrates.

**P06.289****Influence of several polymorphisms in the risk of anti-tuberculosis drug-induced hepatotoxicity in Caucasian population**

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Homozygous deletion *GSTM1* (glutathione S-transferase M1) polymorphism, slow *NAT2* (N-acetyltransferase 2) acetylator genotypes and c1/c1 *CYP2E1* genotype increase the risk of antituberculosis drug-induced hepatotoxicity in Asian population. There are no studies about these polymorphisms and the risk of antituberculosis drug-induced hepatotoxicity in Caucasians.

Case-control prospective study of tuberculosis patients treated with isoniazid, pyrazinamid and rifampin. Cases were patients with antituberculosis drug-induced hepatotoxicity and controls patients without this complication. After DNA extraction from periferic blood, *GSTM1* and *T1* null polymorphisms were performed by PCR and *NAT2* slow polymorphisms and *CYP2E1* c1 and c2 polymorphisms by PCR and RFLP.

We included 38 cases and 60 controls without differences in age, sex, BMI and basal transaminases values. The homozygous deletion at *GSTM1* locus was present in 34.3% cases and 41.7% controls ( $P = 0.47$ ) and at the *GSTT1* locus in 48.6% cases and 26.7% controls ( $P = 0.03$ ). Slow *NAT2* acetylator genotypes were present in 65.8% cases and 61% controls ( $P = 0.67$ ). The 6\*/7\* genotype was only present in cases ( $P = 0.02$ ). *CYP2E1* c1/c1 genotype was present in 86.8% cases and 87.3% controls and c1/c2 genotype in 13.2% cases and 12.7% controls ( $P = 0.95$ ). The *CYP2E1* c2/c2 was not found in any patient. We did not find any relation between *NAT2* slow genotypes and *CYPE1* c1/c1 genotype and the risk of antituberculosis drug-induced hepatotoxicity.

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**P06.290****DNA diagnostics in tuberous sclerosis - development of reliable and economical test**

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Tuberous sclerosis (TSC) is inherited autosomal dominant disorder with incidence of up to 1/5.800 births. Almost 80% of cases are new mutations. Extreme clinical variability complicates diagnostics and genetic counselling. Accurate diagnosis is essential in order to detect and treat a life threatening neurological, renal and cardiac lesions and for prenatal diagnosis purposes. Mutations in TSC1 and TSC2 tumor-suppressor genes were shown to be responsible for development of TSC. The TSC2 gene on 16p13.3 spans over 41 exons, coding for tuberin and the TSC1 gene on 9q34 has 21 exons coding for hamartin. No „hot-spot“ with more frequent mutation occurrence was found. Presented study is a project of bilateral Czech-Greek cooperation in research and development, ME 923. The aim is to establish a comprehensive genetic test for the analysis of TSC mutations in Greek and Czech patients. Greek team is introducing direct sequencing of TSC1 and TSC2 coding sequences what is recent trend, though quite expensive. Czech team is performing preliminary screening for mutations by DGGE and LOH test and MLPA for larger rearrangements. Especially the utilization of LOH test simplifies and accelerates TSC diagnostics in some cases. Czech and Greek sample files are exchanged and examined in the other laboratory. The reliability of DGGE was tested on positive samples from partners' lab. Sensitivity of DGGE was proved on artificial mosaics, revealing 10% mutant lineages. The knowledge acquired from the research collaboration should contribute significantly to the development of valid diagnostic methods at a minimum cost.

**P06.291****Polymorphism of TNFA gene promoter region and chronic inflammatory disorders**

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The analysis of genotypes and alleles distribution for polymorphisms -238G/A in patient groups and in population has not revealed significant differences.

For Crohn's disease the -308A allele of the TNFA gene was detected in 27% of patients but only in 6.8% of population ( $p<0.0001$ ). Similar results were obtain for patients with ulcerative colitis and with asthma (19%,  $p=0.015$  and 32%,  $p<0.0001$ , respectively). The distribution of genotypes for -308 G/A polymorphism was also different in studied groups compared to population. The frequency of combined genotype -238A/G + -308 G/G of the TNFA gene which is associated with the lowest level of gene expression considerably decreased in patients with chronic inflammatory disorders.

Thus, the polymorphism in promoter region (-308 G/A) which is associated with increased expression of the TNFA gene might be considered as an inherited risk factor contributing to development of multifactorial inflammatory diseases.

**P06.292****Gene copy number analysis of complement genes in type 1 diabetes**

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The major contributor to type 1 diabetes (T1D) is located in the MHC complex, and HLA DR3 and DR4 confer maximum risk. Other genes also associated with T1D include INS, CTLA4, PTPN22 and innate im-

une response genes. Differential activation of the complement genes could alter the innate response and contribute to T1D development. These quantitative changes could be caused by copy number variations, a recently described polymorphism type that has been associated with autoimmune diseases.

Our aim was to determine whether gene copy number variants of complement genes C2 and C4B are associated with T1D.

We analyzed 60 T1D patients and 60 nondiabetic control individuals. C2 and C4B copy number quantitation was performed by real-time PCR using specific Taqman assays. Experiments were performed in duplicate using 20ng of genomic DNA. Raw data were normalized using the endogenous RNaseP gene and expressed relative to a reference sample, using the  $-2\Delta\Delta Ct$  method. Comparisons between the two groups were performed using a Student's T test.

T1D patients presented a significantly ( $p<0.01$ ) increased abundance in C2 (mean relative values  $\pm$  SD =  $1.17 \pm 0.58$  in T1D vs.  $0.94 \pm 0.31$  in controls) and a decrease in C4B ( $0.47 \pm 0.43$  vs.  $0.91 \pm 0.45$ , respectively).

We conclude that these differences in gene copy number of complement genes could affect the risk to develop T1D, and these polymorphisms seem to be associated with genetic susceptibility to T1D. However, we have to take into account that these associations could be due to linkage disequilibrium with the HLA class alleles and not to a primary effect.

**P06.293****Polymorphisms in NOS1(C/T), NOS3 (VNTR4a/b, 894G/T), IL4RA (I50V) genes are associated with microvascular complications in patients with type 1 diabetes**

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Type 1 diabetes (T1D) is a multifactorial autoimmune disease. T1D patients face the risk of late diabetic microvascular complications: retinopathy (DR), nephropathy (DN) and polyneuropathy (DP). Genetic polymorphisms genes for NO-synthases and cytokines could be risk factors of vascular damages in diabetes. The aim of this study was analysis of association of candidate genes polymorphisms with diabetic microvascular complications (DR, DN, DP) in groups of T1D patients with DR (n=41), DN (n=60), DP (n=39), combination of DR, DN and DP (n=36) and their families (n=110). The following polymorphisms were studied: NOS1(C/T), NOS3 (-691C/T, VNTR4a/b, 774C/T, 894G/T), IL1B (+3953 A1/A2), IL1RN (VNTR), IL4 (G/C 3'-UTR), IL4RA (I50V). Using Transmission/Disequilibrium Test (TDT), we found following associations: IL4RA allele I and DR (TDT=4.22,  $p=0.0498$ ); NOS3 allele B (VNTR4a/b), allele G (894G/T) and IL4RA allele I with DN (TDT=4.50,  $p=0.0340$ ; TDT=4.32,  $p=0.0376$ ; TDT=4.97,  $p=0.0259$ ; respectively); NOS1 allele C, NOS3 allele B (VNTR4a/b) and IL4RA allele I with DP (TDT=4.69,  $p=0.0303$ ; TDT=4.50,  $p=0.0339$ ; TDT=3.84,  $p=0.0500$ ; respectively); IL4RA allele I with combination of DR, DN and DP (TDT=4.27,  $p=0.0389$ ). Thus, NOS-genes and cytokines genes are the risk factors for T1D and its microvascular complications. Further studies should be conducted to address the molecular basis for such effect.

**P06.294****Presence of additional T1DM risk determinants on HLA-DR3 conserved extended haplotypes (CEH)**

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Type 1 diabetes mellitus (T1D) is a complex autoimmune disease in which insulin-producing pancreatic  $\beta$  cells are destroyed by an aberrant immune response. The major T1DM susceptibility locus is located in the HLA region on chromosome 6p21, and DR3-DQ2 and DR4-DQ8 are the most predisposing haplotypes. Among Caucasians of Southern European origin, DR3/3-conferred predisposition is comparable to DR3/4 risk, probably due to the relative abundance of the extraordinarily homogeneous B18-DR3 CEH in this population, and support the presence of additional determinants within this CEH, that are absent

from other less T1D-predisposing DR3 chromosomes (like B8-DR3 CEH).

To test this hypothesis, we genotyped a panel of 2,360 SNPs in the MHC region, spanning 4.9 Mb (MHC Panel Set - Illumina Inc, San Diego, CA) in 21 T1D patients and 39 non-diabetic individuals who were homozygous for HLA-DR3 and carried only one copy of the complete B18-DR3 CEH (HLA-A\*30-B\*18-DR3-DQ2). Genotype and allele frequency calculations and association analyses were performed using PLINK v.1.0 (<http://pngu.mgh.harvard.edu/purcell/plink/>).

After stringent correction for multiple testing (Bonferroni) six independent SNPs remained associated (uncorrected p-value:  $1.03 \cdot 10^{-5}$  and  $2.73 \cdot 10^{-7}$ ).

Our results add further evidence for the presence of additional susceptibility determinants in the MHC. Since the associated markers might just be proxies of the etiological variants in B18-DR3 haplotypes, we are genotyping these SNPs in HLA-unmatched cases and controls. Further in-depth analysis of these associated regions might be necessary to identify the etiological variants.

#### P06.295

##### Family-based analysis of tumour necrosis factor and lymphotxin alpha tag polymorphisms and type 1 diabetes in the population of South Croatia

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Type 1 diabetes is an autoimmune disease characterized by destruction of pancreatic β cells. It is influenced by environmental and genetic factors. TNF and LTA are cytokines with a wide range of inflammatory and immunomodulatory activities possibly related with type 1 diabetes. The aim of the present study was to evaluate the association of 11 TNF/LTA tag polymorphisms in 160 type 1 diabetes trio families from South Croatia. Investigated tag polymorphisms were designed to capture the majority (62%) of common variation in TNF/LTA gene region, based on the HapMap database. We observed overtransmission of alleles from parents to affected child at four variants: rs909253, allele C,  $p=1.2 \times 10^{-4}$ ; rs1041981, allele A,  $p=1.1 \times 10^{-4}$ , rs1800629 (G-308A), allele A,  $p=1.2 \times 10^{-4}$  and rs361525 (G-238A), allele G,  $p=8.2 \times 10^{-3}$ . We also identified overtransmission of the rs1800629(G-308A)-rs361525(G-238A) G-A haplotype,  $p=2.384 \times 10^{-5}$ . The present study found an association of TNF/LTA gene region with type 1 diabetes. A careful assessment of TNF/LTA variants adjusted for linkage disequilibrium with HLA loci is needed to further clarify the role of these genes in type 1 diabetes susceptibility.

#### P06.296

##### Polymorphisms of the NOS2, TNFB and TNFR1 genes are associated with Type 1 Diabetes Mellitus in Tatars and Russians from Bashkortostan

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Apoptosis may play a role in two different aspects of autoimmune disease - generation of autoreactive cells and tissue destruction. Type one diabetes mellitus (T1DM) is a typical autoimmune disease. The aim of the present study was to investigate the association between apoptosis genes (NOS2, TNFB and TNFR1) polymorphisms with T1DM in Tatar and Russian ethnic groups (Bashkortostan, Russia).

We studied 254 T1DM patients and 544 controls. DNA was isolated by phenol-chloroform extraction from 8 ml venous blood and used as template in PCR-RFLP. Two-tailed test of Fisher was used for statistical analysis.

We have found that NOS2\*G/G genotype was associated with decreased risk and \*G/A genotype was associated with increased risk of T1DM in Tatar males (OR=0.50, P=0.02, CI: 0.28-0.88 and OR=2.18, P=0.011, CI: 1.22-3.87, respectively).

Carriers of TNFB \*A/A genotype had lower risk of T1DM in Tatars (OR=0.56, CI=0.36-0.87). At the same time, TNFB \*G/G is associated with higher risk of T1DM (OR=2.52, CI=1.33-4.79) in Russian ethnic

group.

We have shown that TNFR1\*G/G genotype was less frequent among patients with T1DM ( $15.5 \pm 2.2\%$  vs.  $26.1 \pm 2.8\%$ , P=0.020). We may conclude that TNFR1\*G/G genotype is associated with decreased risk of T1DM in Tatars (OR=0.52, CI: 0.30-0.90 and OR=0.73, CI: 0.54-0.99).

#### P06.297

##### Region wide association study on chromosome 10q25.1-26.3 and its contribution to type 2 diabetes susceptibility in Japanese

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Several linkage studies indicated that human chromosome 10q is one of a candidate susceptibility locus for type 2 diabetes (T2D). But there is no information about certain variant(s) or gene(s) have strong effects on the disease within the region. In order to identify T2D disease susceptibility genes in Japanese, verified SNPs from the databases with a minor allele frequency larger than 0.15 were applied to 10 intervals across a 25 Mb region on chromosome 10q25.1-26.3. Using the 993 SNPs for genotyping a two-stage case-control analysis was applied to Japanese subjects consisted of 888 T2D patients and 898 control subjects. Haplotype and linkage disequilibrium (LD) were assessed based on SNP genotypes of all study subjects. To search for new SNPs in the detected significant gene, screening of exons and 3'UTR are performed in 32 randomly selected T2D patients. We detected 10 SNPs (six intronic and four 3'UTR) in DOCK1 (detector of cytokinesis 1) gene were showed replicated association in the two set of independent DNA samples. These nominal significant SNPs were located in two different Linkage disequilibrium (LD) block containing exons 7-23 and exons 51-52 respectively. When the two data were combined, the most significant association was observed with SNP 827 in 3' UTR of DOCK1 gene ( $p=0.0005$ ). Real time quantitative revealed that the expression of DOCK1 was rather ubiquitous with relatively abundant expression in the pancreas, kidney and brain in T2D patients. Our region-wide association analysis suggests the strong impact of DOCK1 gene with risk of T2D in Japanese.

#### P06.298

##### Study of mtDNA polymorphism in type 2 diabetes patients

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Diabetes is among frequent endocrine diseases. Endocrine system is one of energy-dependent systems in human organism. Phylogenetic mtDNA haplogroups possess different common polymorphisms and can mark effects of these variants on predisposition to common diseases. Samples of 119 type 2 diabetes patients (78 women, 41 men) and 134 healthy people were studied (all living in Tomsk region). Mean age in the groups was  $52 \pm 5.5$  and  $47.6 \pm 10.2$  years, respectively. Ultrasound examination, 24-hours monitoring of blood pressure and fasting glucose measure were performed. Comparison of frequencies of some of Europeans haplogroups (H, U, T, J) has uncovered lower frequency of haplogroup T in type 2 diabetes patients as compared to control group (OR=0.14;  $0.02 < OR < 0.66$ ;  $p=0.007$ ). In the group of type 2 diabetes patients this haplogroup was detected only in two cases, whereas in control it corresponds to known frequencies for European population (11.6%). Our previous study which was conducted on patients with arterial hypertension has shown higher prevalence of haplogroup T in group of patients with left ventricular hypertrophy against patients without this complication. These alternative effects of mitochondrial haplogroup T may reflect different adaptive advantages for an individual carrying the haplogroup, in respect to different conditions. The findings suggest that particular mtDNA haplogroup (i.e. set of haplogroup-specific polymorphisms in mtDNA) may have some impact on energy metabolism and may be predisposing or protective factor for some diseases and their complications. The work was supported by Russian Foundation for Basic research (RFBR) grants 07-04-01526, 06-04-08326.

**P06.299****Further evidence for association of the *RGS2* gene with antipsychotic-induced parkinsonism: Protective role of a functional polymorphism in the 3' untranslated region**

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In our previous study using a candidate gene approach we have shown that the *RGS2* gene (regulator of G protein signaling 2) is associated with susceptibility to extrapyramidal symptoms (EPS) induced by antipsychotic drugs (Pharmacogenet Genomics. 2007 Jul; 17(7):519-28). Thus supporting our hypothesis that since regulators of G protein signaling (RGS) play a pivotal role in dopaminergic transmission, genetically based, functional variation could influence therapeutic response to antipsychotic drugs.

To further investigate our previous report we performed a replication study. EPS were rated in U.S. patients with schizophrenia (African-American and Caucasian) treated for at least a month with typical antipsychotic drugs risperidone, olanzapine, or clozapine. Six single nucleotide polymorphisms (SNPs) within or flanking *RGS2* were genotyped. Odds ratios and confidence intervals calculations indicate association of SNP rs4606 with antipsychotic-induced parkinsonism (AIP) in the overall sample and in the African-American sub-sample with the minor allele having a protective effect. Sequence analysis of the *RGS2* gene further indicated that this SNP is biologically meaningful and could have clinical utility.

**P06.300****UCP3 gene polymorphism and cardiac growth in response to 1 year of endurance training**

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Reduced fatty acid utilization and increased oxidative stress both can contribute to the development of cardiac hypertrophy. Left ventricular hypertrophy in endurance-oriented athletes is generally understood to be a limiting factor for improving maximal oxygen uptake (VO<sub>2max</sub>). Cardiac uncoupling protein 3 (UCP3) can serve to protect the heart against lipid-induced oxidative stress, and stimulate fatty acid transport and oxidation. A variant in the UCP3 gene associated with higher mRNA levels has been identified (UCP3 -55C/T). This variant has been associated with reduced risk of type 2 diabetes and obesity. Recently we have shown that -55T allele was overrepresented in highly elite rowers and was associated with high values of VO<sub>2max</sub>. If UCP3 is important for muscle and heart metabolism and can protect against development of LVH, then one might anticipate -55T variant of UCP3 gene to be associated with insignificant cardiac growth (rational adaptation) in response to endurance training. We have tested this hypothesis in the study of elite Russian rowers (n=19, males). UCP3 -55C/T polymorphism was determined by PCR-RLFP. Echocardiography was performed for two times with one year interval. We found that subjects of CC genotype exhibited the greatest cardiac growth (when interventricular septal wall thickness was measured; CC: 3 (1.4) mm, CT: 1 (0) mm, TT: -1 mm; P=0.019), whereas the individuals of TT genotype exhibited the reduction in septal wall thickness. In conclusion, we demonstrate that variation in the UCP3 gene influences cardiac growth in response to endurance training in rowers.

**P06.301****Powerful new methods for whole genome copy number association studies**

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The latest genotyping arrays provide over a million markers across the genome to interrogate copy number variation (CNV), making it possible to perform whole genome copy number association studies. However, the various statistical and computational challenges involved with the analysis of copy number data on such a large scale have pre-

vented such association studies from being completed. Instead, whole genome copy number studies have been limited to paired sample analysis or visual inspection on small numbers of samples. Arguably, due to small sample sizes and the susceptibility of visual inspection to human error, such approaches offer only cursory insights and lack in their effectiveness to uncover CNV associations.

To address these issues, we developed a series of novel methods embodied in a new tool called CNAM. CNAM is capable of efficiently and accurately preprocessing whole genome copy number data for thousands of samples, finding large to single probe CNVs, and performing a range of statistical tests to identify CNV associations.

Discussed methods include accurately extracting log ratio signals, quantile normalization without gender bias to properly normalize against reference populations, optimal univariate and multivariate segmenting based on dynamic programming (Hawkins Segmentation) to determine regions of variation, enhanced Eigenstrat-based principal component analysis to detect and correct for population stratification and batch effects, and association testing using various copy number measures as covariates.

We demonstrate the utility of these methods by presenting preliminary results on public whole-genome data using thousands of case-control samples.

**P06.302****First case of maternal UPD7 and recessive congenital myotonia**

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Autosomal congenital myotonia dominant (Thomsen) and recessive (Becker) are rare non dystrophic disorders due to allelic mutations of the muscle chloride channel gene, CLCN1, located on chromosome 7q35. Both diseases are characterised by muscle stiffness and myotonia, which is based on an electrical instability of the muscle fiber membrane, but they differ clinically by age at onset.

We report on the clinical and molecular data of the first case of a Becker patient carrying two identical mutations because of a maternal UPD of the entire chromosome 7.

The proband is 14-year-old male with involuntarily prolonged contraction of muscles, hypertrophy calves, normal CPK and EMG with dynamic myotonia. Moreover he was reported as having severe growth retardation and a facial anomalies compatible with the diagnosis of Silver-Russell syndrome. Proband's DNA analyses showed homozygosity for a novel mutation leading to a G355R substitution (GGG→AGG). The mutation segregated only from the mother, while the father was not carrier for G355R. Non paternity was excluded using a panel of 15 highly polymorphic markers and one marker for amelogenin. Only markers located on chromosome 7 showed some segregation anomalies from the father. For this reason, a fluorescent microsatellite analysis was performed using 9 polymorphic markers spanning the entire chromosome 7 region. PCR products were analysed by automated sequencer. All the markers showed homozygosity in proband's DNA. Our results clearly allowed us to affirm the presence of a maternal isodisomy of the entire chromosome 7 (matUPD7) in the affected proband.

**P06.303****A genome wide association study reveals SLC2A9 as a major gene for uric acid levels with pronounced gender-specific effects**

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Germany, <sup>7</sup>First Department of Internal Medicine, St. Johann Spital, Paracelsus Private Medical University, Salzburg, Austria, <sup>8</sup>Institute for Community Medicine, Ernst-Moritz-Arndt University, Greifswald, Germany, <sup>9</sup>Central Hospital of Augsburg, MONICA/KORA Myocardial Infarction Registry, Augsburg, Germany. Serum uric acid (UA) levels are correlated with gout and clinical entities such as cardiovascular disease and diabetes. In a genome-wide association study (KORA F3-500K, n=1644), we identified a QTL associated with UA level located on chromosome 4 including 40 SNPs with P-values below the genome-wide significance level. The most significant SNPs mapped within intron 4 and 6 of *SLC2A9* (effects -0.18 to -0.36 mg/dL per copy of the minor allele). These findings were replicated in three independent samples from Germany (KORA S4 and SHIP) and Austria (SAPHIR) with P-values ranging from  $1.2 \times 10^{-8}$  to  $1.0 \times 10^{-32}$ . The *SLC2A9* genotypes in addition showed significant association with self-reported gout. The proportion of the variance of serum UA levels explained by genotypes was about 1.2% in men and 6% in women. Analysis of whole blood RNA expression profiles from a KORA F3-500K subgroup (n=117) revealed a significant association between the *SLC2A9* isoform 2 and urate levels. *SLC2A9* encodes a transporter protein that belongs to class II of the facilitative glucose transporter family. A potential substrate of GLUT9 is fructose, as GLUT9 has the highest similarity with the fructose transporters GLUT5 and GLUT11. Our expression studies allow discrimination between two annotated isoforms of this gene. The significant association with the shorter protein GLUT9ΔN argues for a prominent role of the *SLC2A9* isoform 2 in the regulation of urate concentrations. The association with the isoform 2 suggests an involvement of the protein in urate excretion.

#### P06.304

#### Association of polymorphisms Ala62Thr in ZNF365 gene and Taq I and Fok I in VDR gene with metabolic alterations in adults with urolithiasis from Yucatan, Mexico

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Urolithiasis (UL) is an endemic health problem in Yucatan, Mexico. Hypocitraturia, hypercalciuria and hyperuricosuria are frequent metabolic alterations in UL. Polymorphisms Ala62Thr in ZNF365 gene and Taq I and Fok I in VDR gene have been associated to UL. Polymorphisms might affect the metabolic pathways involved in UL, so we analyzed the association of these polymorphisms with metabolic alterations in adults with UL from Yucatan, Mexico. 169 patients, 59 males (34.9%) and 110 females (65.1%) were analyzed. UL was confirmed by RX and ultrasonography. 24 hours urine excretion of Na, K, Ca, P, Mg, uric acid, oxalates and citrates were determined. Polymorphisms were determined in DNA samples by PCR-RFLPs. Genotypic and allelic frequencies of each polymorphism were compared between patients with metabolic alterations and patients with normal metabolism. Metabolic alterations found were hypercalciuria 16.6%, hyperuricosuria 13.6%, hyperphosphaturia 7.7%, hypomagnesuria 1.2%, hyperoxaluria 24.9% and hypocitraturia 61.5%. Genetic frequencies obtained for VDR were: Taq I (tt 1.2%, tT 30.8%, TT 68.0%), Fok I (ff 20.7%, fF 53.5%, FF 26.0%) and for ZNF365 (AA 8.9%, AG 29.0%, GG 62.1%). Polymorphisms in the VDR gene were not associated with metabolic alterations ( $p>0.05$ ), whereas allele G of ZNF365 gene was associated with hypocitraturia ( $p=0.044$ ). Mean concentrations of K, Mg and citrate in urine excretion were significantly lower ( $p<0.05$ ) in subjects with GG genotype compared to wildtype. Polymorphism Ala62Thr in ZNF365 gene was associated with hypocitraturia, whereas GG mutated genotype was associated with a lower urine excretion of K, Mg, and citrate in the studied population.

#### P06.305

#### Genetics and Clinical Aspects of Usher and Pendred Syndromes in Iranian Population

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Over 440 genetic syndromes that include hearing loss have been described. Syndromic hearing impairment accounts for 30% of pre-lingual deafness. The two most common types are Usher and Pendred syndromes. Up to now 12 different loci and 9 genes have been identified. Also Pendred is characterized by congenital sensorineural hearing loss, goiter (40-60%) and inner ear abnormalities and impaired vestibular function test. The objective of this study was to identify the prevalence of USH loci in deaf-blind patients and PDS gene mutations in Iranian Population.

Thirty USH families and 80 families with autosomal recessive non-syndromic hearing loss, having two or more affected individuals were subjected to linkage analysis using STR markers. The sequencing for mutation screening was performed for the linked families.

Nine out of thirty USH families were linked to the studied USH I and USH II loci which we identified the mutation in three of these families and mutation screening in the other linked families is on the way. Also we were able to link fourteen autosomal recessive hearing impaired families to DFNB 4 locus and nine of them showed different types of PDS mutations. In conclusion 30% of the families with USH syndrome were linked to one of the known loci, however additional study needed to determine the causative genes involve in the other families. We have also been able to determine Pendred syndrome as the second cause of hearing loss in our population. In addition we have determined four novel mutations in SLC26A4 gene in Iranian patients.

#### P06.306

#### Genetic profile of the *PCDH15* gene in probands with Usher syndrome type I

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#### INTRODUCTION

Usher syndrome type I (USH1) is the most severe form Usher syndrome. It is characterized by congenital severe-profound sensorineural hearing loss, prepuberal retinitis pigmentosa and vestibular areflexia. The *PCDH15* gene is implicated in this disorder and it encodes for protocadherin-15, a large transmembrane protein belonging to the cadherin superfamily.

#### OBJECTIVE

The main of this study is to identify the USH1 causative mutations and to evaluate the *PCDH15* implication in this syndrome.

#### PATIENTS AND METHODS

Fifty-six USH1 patients from Spain and USA, which had previously discarded the most prevalent USH1 gene, *MYO7A*, were studied.

The mutation screening was performed by directly sequencing of all 33 exons and intron-exon boundaries from the *PCDH15* gene.

#### RESULTS

In this study, seventeen different pathologic variants and forty-five polymorphisms in the *PCDH15* gene have been found in USH1 patients. Mutations identified include twelve missense variants, three premature stop codons and two splice site changes. These variants have been found in seventeen of fifty-six USH1 patients (30.6%). One patient was compound heterozygous and two patients had a homozygous mutation. One unique pathologic variant was detected in the rest of studied patients.

#### CONCLUSIONS

Out of a total of 112 alleles, 20 of them (17.9%) were considered pathologic in the *PCDH15* gene.

It can be estimated that the *PCDH15* gene is responsible for about 16% of USH1 patients.

Most of studied patients were carriers of only one mutation. Large rearrangements within this gene could explain this fact.

**P06.307****Genome-wide screen for aberrant DNA methylation in human uterine leiomyoma by MS-RDA**

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Uterine leiomyoma are very common benign tumors in women of reproductive age. However the molecular mechanisms of cause and development of these tumors are poor understood. This study is attempted to examine whether aberrant DNA methylation occurred in these tumors. Therefore we carried out a genome-wide screen for aberrant DNA methylation, adopting methylation-sensitive-representational difference analysis (MS-RDA) using normal adjacent myometria as tester and myoma tissue driver. As the results, a total of 192 clones were sequenced, 27 DNA fragments derived from CpG islands (CGIs) were isolated, and seven of them were from CGI in the 5' regions of known genes, which include *CHARC1*, *FAM44B*, *FLJ33655*, *HSUP*, *MLLT3*, *SLC16A1*, and *ZNF96*. Then, methylation statuses of those CGIs were analyzed by methylation-specific PCR (MSP) using five primary samples of human uterine leiomyoma. Aberrant DNA methylation did not observed in seven genes in five human uterine leiomyoma eventually. Though, this study failed to identify aberrant DNA methylation occurring in the human uterine leiomyoma by a genome-wide screen, it dose not exclude that epigenetic modifications of DNA methylation are involved in the cause and development of uterine leiomyomas. A large population of primary samples and more attempts, such as use cell lines or primary monolayer cultures established from tissue samples, are needed be done to develop this issue

**P06.308****Study of the VEGF Polymorphism in HELLP Syndrome patients**

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**Background:** The vascular endothelial growth factor (VEGF) has a critical role in vasculogenesis and vascular permeability in several diseases including preeclampsia. VEGF G+405C, C-2578A and C-460T SNPs are known to be related to VEGF production. We decided to determine the allele and genotype frequencies of VEGF G+405C, C-460T and C-2578A SNPs in healthy pregnant women and HELLP syndrome patients.

**Methods:** The authors introduced a quantitative real-time PCR method for the determination of the three VEGF SNPs. Blood samples were collected from 71 HELLP syndrome patients and 93 healthy controls. **Results:** There were significant differences in the allele and genotype frequencies of VEGF C-460T SNP between the two study groups. The T allele was present in 71.1% in the HELLP group, while in 53.8% in the controls ( $p=0.0014$ ). The TT genotype occurred significantly more frequently in the HELLP group than in the control group (45.1% vs. 21.5%;  $p$  (for genotype frequencies)=0.0011). The TT genotype carriers had an increased risk of HELLP syndrome, which was independent of maternal age and primiparity (adjusted odds ratio (OR)=3.03, 95% confidence interval (CI)=1.51-6.08;  $p=0.002$ ). Although the VEGF G+405C allele and genotype distributions did not differ significantly between the two groups, the CC genotype carriers were also found to have an increased risk for HELLP syndrome after adjustment for maternal age and primiparity (adjusted OR=3.67, 95% CI=1.05-12.75;  $p=0.041$ ). The VEGF C-2578A SNP was not associated with HELLP syndrome.

**Conclusions:** We found that the VEGF -460TT and +405CC genotype carriers have an increased risk of HELLP syndrome.

**P06.309****VEGF A2578C polymorphism is associated with muscle fiber type distribution in athletes**

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There is strong relationship between muscle fiber type distribution and human physical performance. For example, elite weightlifters and sprinters exhibit large percentages of fast-twitch fibers (FT, also known as type II muscle fibers) compared to controls and endurance athletes. FT fibers have comparatively low capillary density and blood flow capacity and low mitochondria content. Vascular endothelial growth factor (VEGF) is important in the basal maintenance of skeletal muscle capillarization and may influence the determination of muscle fiber type distribution. To investigate the question of the influence of VEGF gene polymorphism on the proportion of fibers types of m. vastus lateralis, we have analyzed the muscle biopsies obtained from 21 elite Russian athletes (all-round speed skaters). The immunoperoxidase technique was employed for immunohistochemical identification of myosin isoforms. VEGF gene A2578C polymorphism was determined by PCR-RLFP. Mean percentages of FT fibers were significantly higher in VEGF CC homozygotes than in VEGF A allele carriers (AA/AC - 38.3 (6.2) %, CC - 47.8 (12.4) %;  $P=0.03$ ). Then we determined distribution of VEGF alleles in 60 elite and sub-elite weightlifters and in 1,113 controls. We found that the frequency of VEGF 2578C allele was significantly higher in weightlifters than in controls (58.3% vs. 48.0%;  $P=0.035$ ). In conclusion, VEGF 2578C allele is associated with increased proportion of FT muscle fibers in all-round speed skaters and with elite power athlete status.

**P06.310****VEGF ID polymorphism and peripheral neuropathy susceptibility**

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Vascular endothelial growth factor (VEGF) is a cytokine involved in angiogenesis and in maintaining of neuronal function. The genetic variation in VEGF promoter may be responsible for different neuropathies, including the diabetic one.

The aim of our study was to investigate the relationship between the -2549 I/D VEGF gene promoter polymorphism and diabetic peripheral neuropathy susceptibility.

A case-control study was performed in 60 diabetic patients with peripheral neuropathy and 60 healthy subjects, sex and age matched. After the informed consent was obtained, DNA was extracted from peripheral blood. The VEGF -2549 I/D genotyping were assessed using PCR method.

The distribution of VEGF -2549 ID in both lots respected the Hardy-Weinberg condition. The frequencies of genotypes and alleles in the patients and healthy control groups were compared using the contingency tables. Although the DD genotype and D allele frequencies were higher in diabetic patients than in controls (DD: 33.3% vs 25%, D: 57.5% vs 52.5%) these differences do not reach the statistical power. We also observed that the trend of association increases if only patients with early onset were compared (OR=1.2).

This preliminary study indicates that the VEGF ID polymorphism may be associated with early onset diabetic peripheral neuropathy. A future study with an increased statistical power is necessary to clarify the relationship between this polymorphism and peripheral neuropathy susceptibility.

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**P06.311****Linkage and association study for VEGF serum level in the isolated population of Campora**

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Vascular Endothelial Growth Factor (VEGF) is an angiogenic factor having a key role in both physiological and pathological angiogenesis. In order to identify QTLs influencing VEGF serum levels, we performed a genome-wide linkage analysis using a sample of individuals randomly chosen in Campora, an isolated population in South Italy.

VEGF serum levels were measured using ELISA method in a sample of 656 individuals. 627 individuals out of the 656 were all related through a 3049-member pedigree and genotyped for 1122 microsatellites on

the genome (average marker spacing of 3.6cM). The heritability for VEGF serum levels was estimated to 0.86 after adjusting for age.

To perform linkage analysis we broke the large genealogy using GREFFA method through a multiple splitting approach recently proposed by Bellenguez and coll.

With the regression-based linkage statistic, we detected a strong linkage on chromosome 6p12.3 (LOD=10.08) at marker D6S459. This linkage signal exactly corresponds to the location of the VEGF gene. Hence, coding and regulatory regions of VEGF gene were sequenced in a sample of 42 individuals. 31 polymorphisms were identified, 22 of which having a MAF>0.05.

The association between these 22 SNPs and VEGF levels was tested using GTAM test, that also corrects for relatedness between individuals through the genealogical information. After correction for the number of independent tests performed, significant association was detected with two SNPs ( $p<0.001$ ) and VEGF serum levels.

Our data indicate that polymorphisms located in the regulatory regions of VEGF gene are significantly associated with VEGF serum levels in Campora population.

#### P06.312

##### Genetic polymorphisms in patients with vesico-ureteric reflux

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Primary congenital vesico-ureteric reflux (VUR) is a very common urogenital tract disorder. It has a strong genetic component which is assumed to influence the development of the disease as well as its progress. So far different genes were analyzed and also several polymorphisms were tested for association with VUR with mixed results. This study presents association analysis for several polymorphisms from candidate genes of interest.

Genotyping was done in 170 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. Allele specific polymerase chain reaction as well as PCR-RFLP was used for analysis. Detection was performed with agarose gel electrophoresis.

Following polymorphisms were included in the study: M31R, S275T, C335R from the gene IL8RA, G-800A, T-509C, L10P, R25 and T231 from the gene TGFB1, rs2229773 from the gene RARA and D299G from the gene TLR4. Allele frequencies were not significantly different between patients and normal controls.

The results of our study indicate that polymorphisms from genes IL8RA, TGFB1, RARA or TLR4 are not associated with the development primary congenital vesico-ureteric reflux. Therefore it is unlikely that these particular variations play a role in the development of this disease.

#### P06.313

##### Vitamin D receptor gene polymorphisms and vitamin D deficient rickets in Turkey

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In Turkey, considerable number of children suffer from vitamin D deficient rickets, which causes growth retardation, impaired bone formation and fracture risk. To explore the association between vitamin D receptor gene polymorphism and vitamin D deficient rickets, we analyzed VDR gene polymorphisms (FokI, TaqI and ApaI) in 24 Turkish vitamin D deficient rickets patients and 100 healthy controls. We showed that "A" (ApaI) allele is more common in patients (83%,  $p=0.002$ ) but there were no significant differences for FokI ( $p=0.693$ ) and TaqI ( $p=0.804$ ) allele frequencies between patients and controls. We also found that the frequency of Tt and Aa genotypes were significantly decreased in patients. This is the first report that demonstrated VDR gene polymorphisms might be an important factor for genetic susceptibility to vitamin D deficient rickets in Turkish population.

#### P06.314

##### Identification of two new mutations in the VLGR1 and the PDE6B genes segregating in a Tunisian family

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Autosomal recessive retinitis pigmentosa (ARRP) is a genetically heterogeneous disorder. ARRP could be associated with extraocular manifestations that define specific syndromes such as Usher syndrome characterized by retinal degeneration and congenital hearing loss (HL). The Usher syndrome typeII (USH2) subtype associates RP and mild to moderate HL with preserved vestibular function. At least three genes USH2A, the very large G-protein coupled receptor VLGR1 and WHRN are responsible for USH2 syndrome.

Here, we report on the segregation of non-syndromic ARRP and USH2 syndrome in a consanguineous Tunisian family, which was previously used to define USH2B locus. Upon extending this family, the genotyping with additional STR markers excluded linkage to USH2B locus. In addition, ophthalmologic reexamination showed phenotypic heterogeneity. We therefore reanalyzed the extended family with regard to the co-occurrence of two different pathologies i.e non-syndromic ARRP and USH2 syndrome. Surprisingly, linkage analysis disclosed the cosegregation of the USH2 phenotype with D5S428 and D5S618 markers corresponding to the USH2C locus while the ARRP perfectly segregates with PDE6B flanking markers D4S3360 and D4S2930. Molecular analysis revealed two new missense mutations p.Y6044C and p.W807R occurring in VLGR1 and PDE6B genes, respectively. Both mutations were absent in 95 genetically unrelated controls. Finally, the combination of molecular findings for VLGR1 and PDE6B genes enable us to explain the phenotypic heterogeneity in particular the severe ocular affection first observed in one USH2 patient. This report presents an illustration of how consanguinity could increase familial clustering of multiple hereditary diseases within the same family.

#### P06.315

##### MLPA in Diagnostics of Copy Number Variations in X-linked Mental Retardation

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X-linked mental retardation (XLMR) is highly heterogeneous. Because of the low prevalence of mutations in known nonspecific XLMR (MRX) genes the systematic screen is not feasible in a routine practice. It is important to introduce new techniques which enable to screen many loci.

MLPA (multiplex ligation dependent probe amplification) is such a technique and it allows a relative quantification of up to 45 different sequences in one reaction. In our study we used the MLPA kit containing probes for 14 different MRX genes to search for copy number variations in a group of 106 XLMR patients. 68 of them were from potential XLMR (pXL) families with two males in different generations affected, and 38 from families with two or more brothers affected (BP).

We detected three variants: two deletions (IL1RAPL1 and PAK3) and one duplication (IL1RAPL1), all in the pXL patients. The duplication was also present in two affected maternal uncles of the proband. All these rearrangements are being confirmed by other molecular (PCR) or cytogenetic (FISH) techniques. The clinical evaluation of all affected patients from the families is in progress.

The frequency of copy number variations in our studied group was 2.8%, but if we consider only pXL patients it becomes as high as 4.4%. We recommend MLPA as a screening for microduplications and micro-

deletions in a large proportion of known MRX genes. Because of its low cost and sensitivity it is suitable for routine diagnosis of X-linked mental retardation.

#### P06.316

#### The chromosome abnormalities, Y-chromosome microdeletions and CFTR gene mutations testing of infertility man from Western Ukraine

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The most widespread genetic factors causing male infertility include different quantitative and structural chromosome abnormalities, Y-chromosome microdeletions and CFTR gene mutations.

DNA and cultivated leukocytes from peripheral blood of 150 males with spermatogenesis failure were analyzed.

Cytogenetic analysis revealed Klinefelter's syndrome - 47, XXY (7 persons), de la Chapelle syndrome - 46, XX (3), disomy of Y-chromosome - 47, XYY (1). Totally quantitative and structural chromosome abnormalities were found in 13 males (9%).

Any changes in the structure of Y-chromosome hadn't been found in males with Klinefelter's syndrome. In patients with 46, XX karyotype whole sequence of AZFa, AZFb and AZFc is absent. Among 3 patients with de la Chapelle syndrome SRY gene had been detected in 2 persons.

We detected various cases of AZF gene subregions deletions: AZFa (1 patient), AZFb (1), AZFb+c (1), AZFc (6). Whole absence of PCR - product of AZFa, AZFb and AZFc and SRY gene was detected in male with azoospermia and normal karyotype that requires next sequencing analyses. Thus, in 7% of examined infertile males with normal karyotype Y-chromosome microdeletions were found. In regard to the group of examined infertile males the percentage of Y-chromosome microdeletions reached approximately 10%.

The molecular-genetic analysis of CFTR gene mutations and IVS8polyT polymorphic locus revealed F508del mutation in 4 persons, 5T polymorphic allele - in 12 males with spermatogenesis failure.

The studies point on high effectiveness of the complex of cyto- and molecular-genetic researches in males with impaired fertility and state their necessity in diagnose.

#### P06.317

#### ZNF750 in psoriasis

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We have previously shown that a dominant mutation in ZNF750 underlies seborrhea-like dermatosis with psoriasisform elements. To assess the contribution of ZNF750 to the pathogenesis of bone fide psoriasis, we screened 250 psoriasis patients from the U.S. for mutations in the 3 exons of ZNF750, the intron-exon boundaries and 1000bp upstream sequences. While no mutations were found in the coding region, a heterozygous ZNF750 5' UTR variant (+46 C>T) was seen in 3 unrelated psoriasis patients. This change was not found in 300 healthy control samples. Our data suggest that the ZNF750 5' UTR variant (+46 C>T) might be associated with psoriasis

#### P06.318

#### $\beta_2$ -adrenergic receptor (ADRB2) polymorphisms in the childhood asthma

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Gene polymorphisms of ADRB2 are candidate gene of genetic predisposition to bronchial asthma (BA) and allergic diseases. From data of literature the genetic variants of cellular receptors possibly influence on efficiency of action of antiasthmatic drugs.

The aims of our study was to investigate genotypes and alleles frequency of two common polymorphisms of ADRB2 gene (Arg16Gly and Gln27Glu ) in boys and girls with different severity BA.

Patients and methods: 160 Caucasians children, 129 boys (81%) and 31 girls (19%) with the set diagnosis of bronchial asthma of different severity were included. Mild asthma was diagnosed in 10 children (6%),

75 children (47%) had moderate asthma and 75 children (47%) had severe asthma. Two common ADRB2 polymorphisms 16 (Q→E) and 27 (G→R) were detected by PCR method with the subsequent restriction assay.

Results: we have revealed association between distribution of haplotypes ADRB2 and severity of BA. Patients with the severe asthma had significantly more frequent haplotype EE/GG than patients with moderate asthma ( $p=0.024$ ) in which more frequent where was haplotype QQ/GR. In group of boys we detected analogical changes ( $p=0.017$ ), but there were no differences in girls.

#### P06.319

#### No significant role of the FXII 46C→T mutation in Spanish and Tunisian cardiovascular patients

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**Background.** - The 46C→T polymorphism in the Coagulation Factor XII gene consists of a cytosine to thymidine transition at the position 46 from the transcription initiation point in exon 1 of the 5'-untranslated region. Previous case-control studies have indicated an association between this polymorphism and variation in plasma levels of FXII. The 46C→T seems to affect the translation efficiency, leading to reduced plasma levels of the protein. It has been inferred that this mutation accounts for 40% of the variance in FXII activity levels in a Spanish Mediterranean population. As other case-control studies in the same region indicate, genotype T/T is an independent risk factor for venous thrombosis, ischemic stroke and acute coronary artery disease (CAD).

**Aim.** - In this study, we try to confirm the importance of the 46C→T polymorphism in two patient samples from Spain and Tunisia.

**Methods.** - We have implemented two different approaches: a Transmission Disequilibrium Test (TDT) based on 101 families (N=302) from Spain (Barcelona) with one offspring with CAD; and a classical case-control study based on 76 patients with CAD complicated by myocardial infarction and on 118 healthy individuals from North and Centre-South Tunisia. All subjects were genotyped for the 46C→T polymorphism with Real-Time PCR using a TaqMan® SNP Genotyping Assay (Applied Biosystems) and the appropriate software was implemented for the data analyses.

**Results.** - No statistically important association was detected in any of the two samples (TDT:  $p=0.16$ , statistical power = 99%; case-control study:  $p=0.65$ ).

**Conclusion.** - The results suggest that 46C→T is not a risk factor for CAD in any of the two analysed samples. So far as we know, the TDT analysis for this mutation is the first one carried out in a Spanish population and suggests that 46C→T might not be the only functional site that would explain the susceptibility to thrombotic disease.

#### P06.320

#### Bsml, Apal and Taql Polymorphisms of Vitamin D receptor gene in Turkish nephrolithiasis patients

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Nephrolithiasis is a multi-factorial disease influenced by genetic, hormonal and environmental effects. Calcium oxalate (CaOx) is the most common constituent of kidney stones. Vitamin D receptor (VDR) involves in controlling the effects of 1,25dihydroxyvitaminD<sub>3</sub>. The 3'UTR region of the VDR gene includes a cluster of polymorphisms: Bsml, Apal and Taql. These polymorphisms have been studied in patients with urolithiasis, but yielded inconclusive results. Since the contribution of alleles may appear to be different in different ethnic populations, the relationship of the individual and combined polymorphisms should be studied for each population. In this study, we investigated the association of Bsml, Apal and Taql polymorphisms in 98 Turkish CaOx stone patients and 70 controls. 52% of the patients reported a family history of stones. The polymorphic sites were amplified with PCR, subjected to restriction enzyme digestion and analyzed on agarose gels. Associations between the disease and genotypes were assessed by calculating odds ratios and 95% confidence intervals. VDR genotype distribution was compared with that of controls using the chi-square

test. VDR genotype frequencies were in Hardy-Weinberg equilibrium. We also carried out haplotype analysis as the polymorphisms are inherited together. In patients, six different haplotypes were defined, with over-representation of BA<sub>T</sub> (40.7%). On the other hand, baT haplotype was the most frequent in controls. BaT haplotype was four times and bAt haplotype was two times more frequent than in controls. This study also provides evidence that there is a statistically significant association of the bT VDR haplotype and formation of CaOx kidney stones.

#### P06.321

#### Changes in Ca homeostasis lead to electrical and structural remodeling of the heart in the 'human PLN' mouse mode

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Phospholamban (PLN), the reversible inhibitor of SERCA2, is a key regulator of calcium homeostasis and cardiac function, and it has been directly implicated in the development of dilated cardiomyopathy. Its amino acid sequence is highly conserved across species except for humans, where Asn is replaced by Lys at amino acid position 27. To evaluate the significance of this single nucleotide difference we induced cardiac-specific insertion of the human-PLN in the null background. The "humanized" PLN expressing transgenic (TG) mouse hearts presented increased inhibition of SERCA2, abnormal calcium handling, fibrosis, and hypertrophy. Using whole genome microarrays (Codelink), we identified significant changes in ion transport, muscle contraction, cell cycle and proteolysis, as well as numerous transcription related genes. The observed changes in key sodium, potassium and calcium plasma membrane pumps were confirmed at the protein level and suggested an ongoing electrical remodeling process. In support to these findings, ex vivo Langendorff perfusion of intact hearts revealed decreased rates of contraction and relaxation in TG mice. Furthermore, patch clamp analysis of isolated cardiac myocytes unveiled significantly prolonged cardiac myocyte action potential, decreased transient outward current (I<sub>to</sub>) and increased sodium/calcium exchanger activity. Significant changes were also detected in key cytoskeletal and contractile proteins. In conclusion, "human-PLN" directly affects calcium cycling and contractility, which in turn triggers electrical and structural remodeling. These compensatory mechanisms ultimately enable long term survival of the TG mice.

#### P06.322

#### The Human and Canine Dopamine D4 Receptor Genes

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The human dopamine D4 receptor (DRD4) is a candidate gene of great interest in molecular studies of human personality and psychiatric disorders. This gene is unique in having an exceptionally high amount of polymorphic sites both in the coding and in the promoter region. One of the most thoroughly investigated polymorphisms is the 48 base pair variable number of tandem repeats in exon III that has been carefully studied as a possible genetic risk factor for several psychological traits and psychiatric disorders, such as novelty seeking, drug abuse and attention deficit hyperactivity disorder.

Mice or rat possess no analogous repeat sequences, whereas similar tandem repeat polymorphism of the DRD4 gene was identified in dogs and chimpanzees. Therefore, genotyping method was developed for exon III length polymorphism of DRD4 gene in dogs; genotype and allele frequency values were measured in several dog breeds, in wolves and in human DNA samples.

Moreover two other VNTRs were identified and analyzed in dogs in exon I and intron II of the DRD4 gene and were found to be polymorphic.

Our results show that DRD4 gene is also variable in dogs, which means that these animals have the potential to become a natural model for testing relationships between this candidate gene polymorphism and psychiatric disorders (such as hyperactivity).

#### P06.323

#### Expression profiles of non-syndromic thoracic aortic aneurysms

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Thoracic aortic aneurysms occur when the arterial wall is unable to resist the dilating force of arterial pressure. Aortic aneurysms tend to expand without symptoms until aortic rupture or dissection take place. The aim of the study has been to define the molecular circumstances in which non-syndromic aneurysm of thoracic ascending aorta can develop.

Fragments of human ascending thoracic aortas with (35, cases) and without (22, controls) aneurysms have been isolated during surgical intervention (aortic/cardiac transplantation or aortic valve replacement). The three layers of each isolated aortas have been dissected. We now report on media layer results.

Gene transcription and proteomic profiles of the aneurysmal compared to non aneurysmal aortic media layer have been studied. Our preliminary oligonucleotide microarray (22000 genes) results evidenced down regulation of genes such as Decorin (DCN), Thrombospondin 1 (THBS1), Reticulocalbin 2 (RCN2), Reticulin 1 and 4 (RTN1 and 4). Paucity of these transcripts delineate a structural lack of resistance to mechanical stress and an increase of angiogenesis possibly as an attempt to remodel the vessel. Proteomic analysis indicated an hyper expression of Testican 2 (SPOCK2) and Jagged 1 (JAG1), confirmed by Real Time PCR analysis: mRNAs for these genes are upregulated. The implication of angiogenesis in the aneurysmal aorta is consistent with upregulated Eph transcripts found in microarray analysis. Preliminary results from microRNA microarray (794 miRNA) analysis indicate differential expression between cases and controls of miRNA 15, 16, 21, 128, 487, and 133, and this is consistent with down and up regulated genes found.

#### P06.324

#### Determination of a novel OA1 gene deletion identified by MLPA in a Spanish family

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Nystagmus is the common symptom of a range of diseases involving the macula, including X-linked disorders like ocular albinism type 1. Prevalence of OA type 1 is estimated around 1 in 50,000 live births in Caucasian populations. The OA1 gene, which has been assigned to the Xp22.3 region and spans approximately 40 Kbp of genomic DNA containing nine exons, encodes a melanosomal membrane glycoprotein G consisting of 404 residues belonging to the protein-coupled receptor family.

A purified DNA sample of a female carrier in a Spanish family suffering from ocular albinism was subject to the p054 probe mix of MLPA, showing a decreased peak corresponding to exon 2 of the OA1 gene. In order to determine whether the OA1 gene was altered, we analysed a DNA sample from an affected male (proband's father), and after MLPA, no peak was observed. Therefore, we focused our sequencing analysis on exon 2 because it appears to be particularly prone to deletions. Here, we report a mutation (g.5815delA) identified in 2 affected males and 4 female carriers of this particular family. This mutation which results in a truncated protein 35 codons downstream, generates a new restriction target site for XcmI, that we further confirmed by digestion of DNA from the 6 affected patients. Indirect approach analysis by using OA-CA, a STR located in intron 1, showed co-segregation with the disease in the family.

In conclusion, the prior screening by MLPA is a suitable molecular strategy for diagnosis of patients suffering from X-linked ocular albinism.

**P06.325**

**Further support for the contribution of the NRG1 gene (8p12-p21) to the risk for schizophrenia: Case-control association study in a German population based on the GRAS Data Collection (Goettingen Research Association for Schizophrenia)**

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**Background:** Schizophrenia is a severe neuropsychiatric disorder with a worldwide prevalence of around 1% and outstanding heritability estimates (80%; Cardno & Gottesman, 2000). The neuregulin 1 gene (8p12-p21) has been strongly associated with an increased risk to develop schizophrenia by numerous studies in several human populations since the first association reported in an Icelandic population (Stefansson et al., 2002). However this prominent genetic risk factor has not been analyzed in the German population so far.

**Methods:** Authors analyzed the genetic variability contained in the «Icelandic core-at-risk» haplotype as well as in the intron 1 of the NRG1 gene in a sample of German origin based on the GRAS Data Collection (Goettingen Research Association for Schizophrenia), made up of 883 DSM-IV-diagnosed schizophrenic patients and 880 healthy controls, in the context of a case-control study. SNPs were genotyped through melting curve analysis in a LightCycler®480 system while microsatellite analyses were carried out in a 3730xl DNA Analyzer®. Haplotype analyses were performed using UNPHASED (Dudbridge, 2006) and PHASE (Stephens et al., 2001).

**Results:** Sliding windows analysis revealed that the frequency of a haplotypic combination defined by markers SNP8NRG241930, 487-2 and D8S1810 (G-12-18) was increased in cases (7.9%) with respect to controls (3.8%), and was significantly associated with an increased risk for the disorder ( $P\text{-value}=7.0\times 10^{-7}$ ;  $\text{OR}=2.21$  95%CI(1.60-3.08)).

**Conclusions:** Our results reinforce the interest in the NRG1 gene as a risk factor with a moderate but robust contribution to the risk for schizophrenia.

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**P06.326**

**Population-based linkage analysis of schizophrenia case-control cohorts identifies a potential susceptibility locus on 19q13**

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Population-based linkage analysis is a novel method for analysing genomewide SNP genotype data in case-control samples that does not assume a common-disease, common-variant model. The genome is scanned for increased identity-by-descent sharing of extended genomic segments within case-case pairs, relative to case-control or control-control pairs. The method is robust to allelic heterogeneity and is suited to mapping genes which contain multiple, rare susceptibility variants of relatively high penetrance. The method has been proposed by Purcell et al. (Am J Hum Genet 2007) and implemented in the software PLINK.

We analysed genomewide SNP genotype datasets for two schizophrenia case-control cohorts, collected in Aberdeen (461 cases, 459 controls) and Munich (429 cases, 428 controls). This analysis must be performed within homogeneous samples and it was therefore necessary to analyse the cohorts separately. Each cohort was first subjected to several procedures to improve genetic homogeneity, including identity-by-state outlier detection and multidimensional scaling analysis.

Using the complete cohorts there was no significant overlap in signals between the Munich and Aberdeen cohorts. However, when testing only those cases with a positive family history of major psychiatric disease, which would fit better with a model of strongly penetrant susceptibility alleles, we saw a distinct peak on chromosome 19q in both samples (spanning the gene APOE but many others too), that appears in meta-analysis ( $P=0.000016$ ) to be around the traditional level for calling genomewide significance for linkage.

**P07. Normal variation, population genetics, genetic epidemiology****P07.001**

**DNA analysis for blood group ABO determination and RhD DNA typing for prenatal diagnosis**

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Blood type group can be determined immunologically or by genetic methods, but genetic methods are more accurate.

We report on a method for ABO and RhD blood type determination, performed by multiplex PCR and PCR-SSP (sequence-specific primers). These analyses provide fast, cheap and easy to perform method for blood type determination. We utilize two PCR reactions for RhD determination - first amplification covers the fragment spanning the intron 3/intron 4 of RhD gene to look for 37bp insert in exon 4. Second PCR is multiplex (3 fragments), it amplifies intron 7 and 3'UTR of RhD locus. Until now we have already done 14 prenatal diagnoses in RhD (-) mothers. The obtained results for the fetuses RhD blood type were: 13 RhD positive and 1 RhD negative.

Determination of ABO blood type includes 4 specific regions analyzed by PCR-SSP. Until now we have tested this method only using control DNA probes to detect the method specificity and sensitivity. The obtained results are promising and the method could be applied routinely for ABO blood group determination. Both methods for DNA blood group/RhD typing will be applied for the purposes of the individual identification and paternity testing, as such analysis is in some cases requested simultaneously with the DNA genotyping of the individuals.

**P07.002**

**The ACE I/D polymorphism in Lithuanian professional athletes**

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<sup>1</sup>Department of Human and Medical Genetics, Faculty of Medicine, Vilnius University, Vilnius, Lithuania, <sup>2</sup>Lithuanian Olympic Sports Centre, Vilnius, Lithuania. Human physical performance is under strong influence of genetic factors. I/D polymorphism in the human angiotensin-1-converting enzyme (ACE) gene characterised by the presence (I allele) or absence (D allele) of a 287-base-pair Alu repeat within intron 16 is among most extensively investigated ones with respect to ACE activity and its involvement in various pathophysiological conditions related to endurance. Nevertheless, the results are still conflicting across studies and populations. In the present study, ACE gene I/D polymorphism was investigated in 413 Lithuanian professional athletes representing four functional groups [endurance (N=57); mixed sports (N=44); strength and speed (N=30), and team sports (N=282)], as well as in 120 samples from general population of Lithuanians. Statistically significantly higher D allele frequencies were found in strength and speed group ( $P=0.02$ ) as well as in endurance group ( $P=0.06$ ), contrary to the prevailing data from other studies showing association of endurance with I allele. D allele also appeared to be more frequent in the general population of Lithuanians (60.4 %) in comparison to the majority of other European populations (30-50%). Thus, increased D allele prevalence in strength and speed group of athletes from Lithuania can be a reflection of population frequency of this allele. In conclusion, our results imply that the role of ACE gene I/D polymorphism in athletic performance is not straightforward and can be masked by other genetic and non-genetic factors.

**P07.003**

**Association of the ACTN3 gene variant with endurance athlete status**

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Alpha-actinin-3 (ACTN3) is a myofibrillar protein found in fast-twitch glycolytic muscle fibers. The less common X-allele of the R577X polymorphism in the ACTN3 gene results in a premature stop codon and alpha-actinin-3 protein deficiency in XX homozygotes. A strong association has been reported between the R577X polymorphism and elite athletic performance. The aim of the study was to investigate genotype and allele distribution of ACTN3 gene in endurance-oriented athletes and controls. The study involved 501 athletes (biathletes; rowers; long

distance runners, swimmers and skaters, road cyclists, skiers, triathletes, race walkers) and 1197 controls. Genotyping was performed by restriction fragment length polymorphism analysis. The distribution of ACTN3 genotypes (athletes: RR - 37.1%, RX - 53.1%, XX - 9.8%; controls: RR - 36.8%, RX - 49.0%, XX - 14.2%) was significantly different between athletes and controls ( $P=0.038$ ). While R allele frequency did not differ between athletes (63.7%) and controls (61.3%), the frequency of XX genotype was under-represented in athletes compared to controls (9.8% vs. 14.2%,  $P=0.013$ ). We therefore conclude that XX genotype is unfavorable for endurance performance.

#### P07.004

#### Analysis of *IGF-1* gene and *PGC-1* gene polymorphism in newborn and elderly people from North-west region of Russia

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Our goal was to investigate whether a polymorphism in the insulin-like growth factor I promoter gene (*IGF-1*, wild-type, 192 base pairs) and in the peroxisome proliferator activated receptor coactivator-1 gene (*PGC-1*, Gly482Ser polymorphism) influence life expectancy.

Different distribution of *IGF-1* (CA repeats) gene polymorphism was shown. Increasing of 20/- genotype in elderly people compared with newborn group (26.7% and 44.1%, accordingly,  $\chi^2=8.57$ ,  $p=0.0034$ ) and decreasing of 19/19 genotype (51% and 27.9%, accordingly,  $\chi^2=14.815$ ,  $p=0.0001$ ) were founded. Furthermore, it was shown different distribution of *IGF-1* (CA repeats) gene polymorphism in man and woman. Increasing of 19/20 genotype in newborn man compared with newborn woman (23.2% and 11.3%, accordingly) as well as significant increasing of 19/20 genotype in elderly man compared with elderly woman (44.4% and 21.1%, accordingly,  $\chi^2=5.009$ ,  $p=0.025$ ) was detected. We suggest a possible role of *IGF-1* gene CA- polymorphism in ageing.

The prevalent Gly482Ser polymorphism of the *PGC-1* gene has not been shown to be associated with life expectancy. Increase of Gly/Gly genotype in elderly woman compared with elderly man (51.0% è 27.3%, accordingly,  $\chi^2=4.063$ ,  $p=0.0438$ ) was registered. Analysis of joint contribution of both genes in ageing revealed the significant difference between groups of newborn and elderly people (20%, 41.8%, accordingly,  $\chi^2=4.858$ ,  $p=0.0275$ ). We suggest that both genes (*IGF-1* and *PGC-1*) could be involved in ageing together.

#### P07.005

#### Allele frequency distribution for 3 microsatellite marker in chromosome 12 in Tehran Lipid and Glucose Study

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Objective To study the distribution of allele frequency of 3 microsatellite marker (D12S96, D12S1632 and D12S329) in chromosome 12 on a representative sample of Iranian population

Methods 534 members of 110 families were selected from Thehran Lipid and glucose Study. The DNA samples amplified by multiplex PCR in ABI thermal cycler. Electrophoresis of amplification product performed on an ABI genetic analyzer. After PCR amplification and separation by electrophoresis, raw data was compiled; analyzed and numerical allele designations of the profiles obtained. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination, and power of exclusion were calculated. Bonferroni's correction performed before each comparative analysis.

Results: Several new alleles (not yet reported in the NIST Short Tandem Repeat DNA Internet Data Base [<http://www.cstl.nist.gov/biotech/strbase/>]) detected in this study. We found 15 alleles for D12S96, 10 alleles for D12S1632 and finally 10 alleles for D12S329. The most heterozygote and informative allele was D12S1632 with 0.7270 heterozygosity (208-230bp) and 0.7395 PIC number. There was no significant deviation from Hardy-Weinberg equilibrium for all the observed loci.

Conclusion: Comparing Iranian data with those obtained from other populations, the most informative allele in this population is D12S1632.

#### P07.006

#### Alpha 1 antitrypsin gene mutation in patients with a suspected alpha 1 antitrypsin protein deficiency

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Alpha 1 antitrypsin (AAT) deficiency is the most prevalent potentially lethal hereditary disease of Caucasians. Individuals with AAT deficiency have an increased risk of early onset for severe pulmonary emphysema and for liver disease. The most common variants of AAT are classified as M, S, and the most prevalent type of clinically important AAT deficiency, the Z genotype.

**MATERIAL AND METHODS:** We sequenced the coding region of the AAT gene in 105 patients with a suspected deficiency of AAT protein. We analyzed the sequences by comparing all exons amongst them and also with the reported AAT sequence in the databases to identify all known and novel allelic variants in the gene. Some patients were also tested for plasma levels.

**RESULTS:** We found eight new genetic variants in the coding sequence of the AAT gene. Two were small deletions and six were SNP's. Some of them changed the amino acidic sequence of the protein and were found in more than one patient. All new genetic variants were found in an heterozygous state.

**CONCLUSION:** Naturally occurring mutations have been very useful in understanding regulation-functionality of proteins. Using the sequencing method for genotyping, has showed to be a very useful tool for discovering new genetic variants. The knowledge of new genetic variants in the AAT gene, specially when they occur in the coding region of the gene, could contribute to identify new genetic risk factors for suffering the AAT deficiency disease.

#### P07.007

#### Phenotyping of alpha-1-antitrypsin in hospitals of three regions of Azerbaijan

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Alpha-1-antitrypsin ( $\alpha$ 1AT) is a low molecular protease inhibitor, synthesized by liver cells and suppressing the activity of many serine proteolytic enzymes. In our experiments for revealing subtypes of normal Pi alleles: M1, M2, M3 and diagnosis of mutated alleles - PiZ, PiS we used analytical method of isoelectrofocusing (IEF) in thin layer polyacrylamide ampholine gels (PAAG), pH 4-6. We have done identification of  $\alpha$ 1AT phenotypes in quite healthy persons as well as in patients with COPD. Capillary blood (0.2 ml) with anticoagulation agent - heparin was collected in eppendorf tubes. Altogether there were screened 919 persons' blood samples in 3 regions : Siyazan and Khachmas areas are located 110 km northward and Khazakh area 450 km west-northward from Baku city. The frequency of mutated gene PiZ and PiS varied in the limits of 0.0046-0.0114 and 0.0037 and 0.0066 respectively. The lowest frequencies PiZ of the gene were revealed at population of Khazakh, PiS gene at the population of Siyazan. Among the patients with COPD from all central region hospitals there were identified the homozygote state for PiZ mutations with phenotypic frequency 0.83% (Khazakh) up to 1.81% (Khachmas) at the average - 1.16%. Only among the patients in Siyazan there was identified compound-i.e. double heterozygote state of PiZ and PiS genes. About the three regions there were revealed 32 mutations of PiZ heterozygote, homozygote - 19 people, mutations of PiS heterozygote - 22, homozygote one and one compound with PiZ/PiS genotype.

#### P07.008

#### Leukocyte and Plasma Alpha-Galactosidase Enzyme Activity Levels in Healthy Young Adults: Evidence that Females Have Higher Plasma Levels

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**Background:** Lysosomal  $\alpha$ -galactosidase is the enzyme deficient in Fabry disease, an X-linked glycosphingolipid storage disorder affecting both genders. Although in males the leukocyte and plasma  $\alpha$ -galactosidase assays are used interchangeably for the diagnosis of Fabry disease, in females the enzyme assays are less reliable and the plasma assay appears to have a much lower sensitivity than the leukocyte assay.

**Methods:** To investigate possible biologic gender differences in normal  $\alpha$ -galactosidase activity, plasma and leukocyte enzyme activities were determined in eight male and nine female healthy unrelated Caucasian young adults. All subjects had been genotyped and carried the standard genomic sequence for the 5' untranslated region of the  $\alpha$ -galactosidase gene, a region that may contain single nucleotide polymorphisms affecting the level of gene expression. Differences in gender means were compared by the independent samples t-test.

**Results:** The mean ( $\pm$ SD)  $\alpha$ -galactosidase activity levels were 28.7 ( $\pm$ 3.7) and 27.5 ( $\pm$ 4.7)  $\mu$ kat/Kg in leukocytes ( $p=0.57$ ) whereas mean plasma activities $\pm$ SD were 2.2 ( $\pm$ 0.5) and 2.9 ( $\pm$ 0.7) nkat/L ( $p=0.03$ ), respectively for males and females.

**Conclusion:** There seems to be a gender difference in normal plasma  $\alpha$ -galactosidase activity. This finding, if confirmed in a larger sample, may have to be taken into account in the definition of normal laboratory reference ranges and may warrant a reassessment of the sensitivity of the plasma  $\alpha$ -galactosidase assay for the identification of females with Fabry disease. We hypothesize that a higher fractional post-translational sialylation of the newly synthesized enzyme in the female Golgi apparatus may contribute to this difference.

#### P07.009

#### Familial amyloid polyneuropathy (ATTR V30M): a change in paradigm?

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Andrade first described FAP (1952) as a disease occurring between 25 and 35 yrs. He reported 64 patients, 13 of which had no family history of the disease. Later PE Becker established its AD mode of inheritance and interpreted isolated cases not as a *de novo* mutation but as the expression of incomplete penetrance of the gene in one of the parents. This hypothesis could only be tested after the finding of the mutation in 1985.

Our aims were: 1) to estimate the number of probands with no affected parent at time of diagnosis; 2) to study age at onset of proband and its changes over time. Between 1939 and 2005, 2075 patients (525 families) were diagnosed at HGSA. Families were classified as "new" when the proband reported no similar disease in earlier generations.

Age-at-onset varied from 20-80 yrs (mean 37.1 in women, 32.4 in men). 209 probands (40%) had no affected parent at time of diagnosis. This type of family represented 68% of those diagnosed in last decade (only 27% before 1985). Mean age-at-onset of probands of "new" families was 46.0 yrs (vs. 32.3 yrs for "classical" families) and it has increased over time, being 49.5 yrs in last decade.

Regarding FAP two realities coexist in Portugal: families with several generations of affected (where probands have "classical" onset) and probands with late-onset who report no similar disease in previous generations. The mutation may cross generations without clinical manifestations, then expresses as late-onset and later anticipation occurs.

#### P07.010

#### The $\beta_2$ -adrenergic receptor gene polymorphism and arterial hypertension in children

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The  $\beta_2$ -adrenergic receptor (*ADRB2*) is thought to be associated with arterial hypertension (AH) and its altered function can result in increased blood pressure (BP). Q/E27 polymorphism of *ADRB2* gene is considered functionally important.

Though some reports indicate that polymorphism in the *ADRB2* gene is associated with essential hypertension, its role in BP levels remains unclear.

The aim of this study was to investigate distribution of *ADRB2* genotypes and allele frequencies of Q/E27 polymorphism in hypertensive and normotensive children and to compare clinical systolic BP levels between carriers of the different genotypes.

84 children with systolic AH (aged 7-17) and 90 normotensive children

(aged 7-17) were included in the study.

Arterial hypertension was defined as systolic/diastolic blood pressure measurements higher than 95 age-gender-height percentile of the adopted reference values.

DNA was extracted from blood samples according to standard protocols. The Q/E27 polymorphism was genotyped by using the PCR restriction fragment length polymorphism analysis.

The distribution of *ADRB2* genotypes and allele frequencies did not differ significantly between patients with AH and control subjects.

Clinical systolic BP in patients with QQ- genotype was  $133.65 \pm 11.5$  mm Hg, QE-genotype was  $133.43 \pm 8.4$  mm Hg, EE- genotype was  $132.52 \pm 7.5$  mm Hg. Clinical systolic BP differences among genotypes were not found.

The allelic and genotypic frequencies of the Q/E27 polymorphism in hypertensive and control subject

Q/E27	Hypertensive (n=84)	Control (n=90)	p
QQ	17 (20,2%)	14 (15,6%)	0,722
QE	46 (54,8%)	52 (57,8%)	
EE	21 (25,0%)	24 (26,7%)	
Q	80 (47,6%)	80 (44,4%)	0,5527
E	88 (52,4%)	100 (55,6%)	

#### P07.011

#### IL4 genetic polymorphisms contribution to Madeira population risk of asthma

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The allergic disease prevalence is influenced by both environmental exposure and the population's genetic predisposition to the disease. Five different polymorphisms of previously associated genes to some of asthma's phenotypic characteristics namely IL4RP2 and IL4-590 (C/T) at 5q31-32; ADRB2 16A/G at 5q31-32; ADAM33 S1 (G/A) and ADAM33 V4 (C/G) at 20p13 were studied in 28 asthmatic children with positive skin prick test to house dust mite from affected sib-pair families composed by two children and their biological parents. Allele frequencies from asthmatic patients were then compared to a sample from Madeira general population. Both genotypic and allelic frequencies as well as comparisons between both groups were accessed by ARLEQUIN 3.1.

We have found significant differences regarding both IL4RP2 and IL4-590 (C/T) polymorphisms between the two groups ( $p<0.05$ ). No significant differences were detected for the remaining polymorphisms.

There seems to be an association between asthma development and both RP2 and -590 (C/T) polymorphisms within IL4 gene for Madeira population. These two loci may be useful genetic markers for detecting atopic asthma disease predisposition in Madeira.

#### P07.012

#### Gene-Environment-Interactions: Increased Risk for Atopic Eczema

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Within the atopic march, atopic eczema (AE) is one of the first clinical manifestations. Possible interaction of genetic disposition and environmental factors "*in utero*" or postnatal could influence the susceptibility for AE.

For a reliable prognosis of an individual responsiveness to environmental factors, children of the epidemiological LISA-study (n=881) were genotyped for common polymorphic xenobiotica-metabolizing enzymes involving in biotransformation of industrial pollutants, cigarette smoke and in defence against oxidative stress.

The study revealed, that children with the genotype combination *CYP2D6*\*1A/\*1A, *GSTP1*\*1A/\*1A, *GSTT1*\*null/\*null and *GSTM1*\*1A/\*null or \*1A/\*1A (n=26) had a higher susceptibility for AE, when ex-

posed to tobacco smoke (mother smoked actively).

For the exposure scenario "mother smokes during the first trimester of pregnancy", the OR for AE within the first years of the children's life are statistically significant (at the age of **1 year: OR 6.4** [95%CI: 1.0-41]; **1<sup>1/2</sup>: OR 9.5** [95%CI: 2.0-43.9]; **2: OR 5.5** [95%CI: 1.2-24.6] as well as **6 years: OR 3.5** [95%CI: 0.8-15.5], adjusted to gender, positive family history of atopy, vaccination and infections and refurbishing during pregnancy). Children without tobacco smoke exposure show no association between the genotype combination and a higher risk for AE. Additionally, "smoking after pregnancy" could confirm the previously association, but only by a trend due to the small subgroup.

These associations show a putative role of the gene-environment-interactions in the manifestation of AE. However, an identification of a closer time-frame of risk toward the exposure "mother smokes" is not yet possible.

#### P07.013

##### Prevalence of mutations in the *OTOF*, *PJVK* and *DDP1* genes in subjects with auditory neuropathy

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Auditory neuropathy (AN) encompasses a variety of disorders characterized by normal otoacoustic emissions (OAE) and absent or grossly abnormal auditory brainstem responses (ABR) in the affected subjects. AN can result from environmental or genetic causes. It can be part of a systemic neurodegenerative disorder or it can be an isolated clinical entity. The primary lesion in AN can be located in the inner hair cells, in the auditory nerve, or in the synapse in between. In the last few years, several genes have been shown to be involved in AN. We have investigated the prevalence of mutations in the genes encoding otoferlin, pejvakin and TIMM8a (*OTOF*, *DFNB59* and *DDP1*, respectively) in subjects with isolated auditory neuropathy. A cohort of 36 unrelated subjects with isolated auditory neuropathy were screened for mutations in these genes by DNA sequencing of all exons and flanking intronic sequences. In 25 subjects (69%), we found two mutant alleles of *OTOF*, two of the mutations being novel. In one subject (2.8%), we found a novel mutation in the *DDP1* gene. Mutations in this gene are responsible for Mohr-Tranebjaerg syndrome, an X-linked condition associating deafness and muscular dystonia. The affected child did not present with dystonia at the age at which the study was performed. No mutations were found in the *DFNB59* gene. Our results show that mutations in the *OTOF* gene are a major cause of inherited auditory neuropathy and suggest that cohorts of apparently isolated auditory neuropathy may contain additional cases of the rare Mohr-Tranebjaerg syndrome.

#### P07.014

##### Specific genetic diagnoses in Autism Spectrum Disorders. A 10-year genetic study of 159 patients from 5 psychiatric outpatient care units

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Since 1998, a collaborative study has been undertaken between the genetic department of Necker Enfants Malades hospital and 5 psychiatric outpatient care units of Île-de-France region. From 1998 to 2008, 159 patients with autistic spectrum disorders (ASDs) have undergone on-site genetic consultation gathering the following data: family and

personal medical history, detailed psychiatric evaluation, dysmorphological and neurological clinical examination. Blood and/or urine samples were collected for genetic testing each time a specific disorder was suggested after the first consultation, or for systematic screening of common ASD-related disorders. Systematic screening comprised high resolution banding karyotype, with 22q13 and 15q11q13 FISH analyses, *FMR1* gene triplet expansion testing, and metabolic screening (*blood aminoacid and urine organic acid chromatographies, AICAR, urinary creatin and guanidinoacetate, N-glycosylation, lactate, pyruvate, ammonium*). CGH-array analysis was undertaken in 6% of patients thus far. Most of the 159 ASD patients (50 females and 106 males) had associated mental retardation ranging from mild to profound.

21 specific diagnoses were obtained for 30 patients: fragile X (8 patients), 15q11q13 duplication (2 patients), 22qter deletion, 22q11.2 deletion, 1p36 deletion, Williams syndrome, trisomy X, Turner syndrome, interstitial 19q deletion, mosaic ring 15, 8q23q24 duplication, 17p11.2 duplication, 1q31 duplication, 1q21 duplication, *AP1S2* mutation, Prader-Willi, Cornelia de Lange, CHARGE and Rubinstein-Taybi syndromes, creatin transporter deficiency (2 patients), GAMT deficiency, phenylketonuria, and histidinaemia. We present a cohort of 159 patients with ASD. 23 specific genetic disorders were found in 32 patients (20,1%). These results emphasize the importance of on site evaluation of these patients.

#### P07.015

##### Analysis of ACE and AT1R gene polymorphisms in Balkan Endemic Nephropathy

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Balkan endemic nephropathy (BEN) is a multifactorial disorder with still unexplained hereditary component. Similarity of BEN and cyclosporine nephropathy suggests possible common ethiopathogenetic mechanisms. Considering the role of renin-angiotensin system in emergence of cyclosporine nephropathy and other types of kidney disease, candidate genes for association studies in BEN were chosen. We performed analysis of I/D polymorphism in gene encoding for angiotensin-converting enzyme (ACE) and A1166C polymorphism in gene encoding for type I receptor for angiotensin II (AT1R). The study was carried out in a group of 50 patients with BEN diagnosis according to criteria of Danilovic, derived from endemic region in Kolubara district. Two control groups consisted of 50 healthy persons (C) and of 45 patients with other nephropathies (NBEN), both matched by age and gender. DNA for gene analysis was extracted from peripheral blood leukocytes. For detection of ACE I/D and AT1R A1166C gene polymorphisms PCR and PCR/RFLPS methods were used, respectively. We found that frequency of ACE DD genotype was 41.66%, 43.14% and 84.61% in BEN, C and NBEN group, respectively. For AT1R A1166C gene polymorphism frequency of CC genotype was 10.41%, 8.69% and 5.72%, respectively. Our results showed no difference in analysed genotypes between BEN and C group. However, we found significantly higher frequency of ACE DD genotype and lower frequency of AT1R 1166 CC genotype in NBEN group. Although these results do not indicate significant role of ACE and AT1R gene polymorphisms in BEN, we will proceed in investigation of other members of renin-angiotensin system.

#### P07.016

##### Compound heterozygosity between HbS and b-thalassemia. Implication for the neonatal diagnostic of hemoglobinopathies

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Compound heterozygosity between Hb S and b-thalassemia is a form of sickle cell anemia (SCA) with a clinical severity varying considerably. The clinical phenotype depend upon the type of thalassemic defect but also on other factors. To clarify the spectrum of b-thal associated with

HbS in the French population, we studied a cohort of about 150 S/bthal patients and we found 20 different thalassemic traits, 9 of them representing 79% of the patients. Spectrum of mutations vary depending on the patients origin but one interesting finding is that in the group of patients of unidentified origin (n = 31) we found the largest spectrum with 20 different mutations. This finding may be explained by the increasing heterogeneity of the French population and the admixture of thalassemic traits from various parts of the world with the HbS. In France, newborns belonging to populations at risk are screened for SCA by means of IEF and CE-HPLC of Hb eluted from a dried blood spot. In order to know the proportion of incorrectly diagnosed newborns we extracted DNA from blood spot and genotyped the codon 6. Results obtained with 100 spots diagnosed as "S/S" with Hb study, revealed that 15% of samples displayed one Hb S allele and one normal codon and thus were improperly diagnosed. These data, lead us to develop a fast and simple method which will allow to provide an "one shot" correct diagnosis of SCA and related syndromes in neonates.

#### P07.017

#### Genetic heterogeneity of β-thalassaemia in Catalonia: molecular characterization of 74 cases

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**Introduction:** A study performed in Catalonia in 1988 showed 8 mutations responsible for the total of β-thalassaemia cases. The objective is to investigate the β-thalassaemia underlying mutations to ensure proper genetic counseling and prenatal diagnosis. We also describe a new β-thalassaemia mutation.

**Methods:** 74 patients for β-thalassaemia were studied. The 8 most prevalent mutations (>90%) of β-globin in the Mediterranean: CD39(C>T), IVS1:110(G>A), IVS1:6(T>C), IVS1:1(G>A), IVS2:745(C>G), IVS2:1(G>A), -87(C>G), CD6(-A) were screened by ASO. Negative samples were sequenced for β-globin gene.

**Results:** Distribution of the identified β-gene mutations and data of 1988 are shown in Table 1.

**Discussion:** Identification of mutation leading to β-thalassaemia by analyzing the most prevalent mutations in the Mediterranean has decreased 15-25%. The 4 mutations most prevalent in our study are common in the West Mediterranean but the last 4 considered prevalent are responsible only for 2.8% of our cases. In comparison with the 1988 study, analysis of the 8 mutations then identified is responsible for 82.6% in the recent study, changing the relative percentage. Molecular heterogeneity has increased: 19 mutations, including Chinese and sub-Saharan variants are now responsible for all cases. Since immigration flows and increased rates of rare and even new variants make genetic diagnosis more difficult, this shows the high importance of identifying β-thalassaemia mutations in order to provide appropriate genetic counseling and prenatal diagnosis

MUTATION	ORIGIN	% 2007	% 1988
<b>Prevalent mutations</b>			
CD39 C>T	17 Spain 1 Italy 1 Russia 1 Morocco 1 Subsaharan	28.4	64.0
IVS1:110 G>A	9 Spain	12.2	8.5
IVS1:6 T>C	6 Spain 5 Egypt	14.9	15.5
IVS1:1 G>A	10 Spain 1 Russia 1 Morocco	16.2	3.5
IVSII:745 C>G	1 Spain	1.4	1.7
IVSII:1 G>A	-	-	-
-87 C>G	-	-	-
CD6 -A	1 Morocco	1.4	5

MUTATION	ORIGIN	% 2007	% 1988
<b>Other mutations</b>			
CD8 -AA	1 Spain 3 Morocco	5.4	1.7
IVS1:5 G>C	1 Spain 1 Morocco	2.7	1.5
CD8/9 +G	1 Spain 1 Morocco	2.7	-
CD41/42 -TTCT	2 China	2.7	-
CD24 T>A	2 Subsaharan	2.7	-
CD6 -AG*	2 Spain	2.7	-
CD37 G>A	1 Spain	1.4	-
IVS1:1 G>T	1 India	1.4	-
IVSII:849 A>C	1 Subsaharan	1.4	-
-29 A>G	1 Subsaharan	1.4	-
-88 C>T	1 Spain	1.4	-

#### P07.018

#### Polymorphism in CaRS A986S gene and serum or urine concentration of bone mineral related parameters in random selected postmenopausal women .

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**Background.** Calcium-sensing receptor (CaSR) is a candidate gene for osteoporosis susceptibility. There are only a limited number of studies available regarding the relationship between the CaSR gene A986S polymorphism and serum or urine concentration of bone mineral related parameters.

**Objectives.** To evaluate whether CaSR gene A986S polymorphism affects bone mineral related parameters on serum as: calcium (s-ca), phosphorus (s-ph), alkaline phosphatase (s-alp), acid phosphatase (s-acp), or on urine as calcium creatinine ratio (u-ccr).

**Subjects and methods.** 158 women with natural menopause were randomly selected (mean age 52.7±1.6 yr). They did not have any diseases to affect bone metabolism. Genomic DNA was extracted from peripheral blood leukocytes by the Higuchi method. A fragment of exon 7 of CaSR gene containing the A986S polymorphism was amplified by polymerase chain reaction (PCR). After amplification, all samples were digested with Bsa H1 restriction enzymes, and the fragments were separated by agarose gel electrophoresis. The levels of s-ca, s-ph, s-alp, s-acp or u-ccr were measured by P-module from Roche.

**Results.** Genotype frequencies of CaSR gene A986S polymorphism was: AA, 121 (76.6%); AS, 34 (21.5%), and SS, 3 (1.9%). We found no association between CaSR polymorphism and s-ca, s-ph, s-alp, s-acp. Women with the AS/SS genotype had higher u-ccr than those with AA genotype, (AS/SS 0.205 ± 0.88; AA, 0.167 ± 0.76 mg/mg; p=0.014 ).

**Conclusions.** 1) We found no association between CaSR gene A986S polymorphism and serum concentration of bone mineral related parameters. 2) We found association between CaSR gene A986S and calcium creatinine ratio.

#### P07.019

#### BRCA1 and BRCA2 sequence variants in healthy women in Croatia

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BRCA1 and BRCA2 are the major genes predisposing to breast and ovarian cancer. Mutations in either of these tumor suppressor genes are associated with both sporadic and hereditary forms of breast cancer. In hereditary cancer, a person inherits one mutated copy of either one of these genes. Tumorigenesis occurs when in addition to having a mutated copy individual develops an inactivating mutation of the remaining healthy allele.

Breast cancer is the most common malignant disease of female population, and the second most common malignancy-related cause of death. At least ten percent of cases are attributable to familial inheritance. In Croatia, more than 2200 new cases of breast cancer are diagnosed each year, and about 800 women die of this malignancy.

The 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.

In total, we analyzed 230 samples for BRCA1 gene and 140 samples

for BRCA2 gene. We found 21 different sequence variants in BRCA1 (2 novel) and 36 variants in BRCA2 gene (7 novel).

We analyzed the distribution and occurrence of sequence variants in BRCA1 and BRCA2 genes on a healthy population of women in Croatia in an attempt to distinguish non-tumorigenic from tumorigenic changes in genomic sequences of BRCA1 and BRCA2 genes. This may contribute to easier distinction of potentially dangerous from harmless changes in patients with family history of breast cancer.

#### P07.020

#### DNA-repair genetic polymorphisms and breast cancer risk among Cypriot women

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Breast cancer is the most common malignancy which affects women worldwide. In an attempt to identify genetic variants which modify breast cancer risk we are contacting a case-control genetic epidemiology study using a cohort of 2286 Cypriot women (1109 breast cancer patients and 1177 age-matched healthy controls). In the present study we genotyped 11 single nucleotide polymorphisms (SNPs) in BRCA2, ERCC2, FANCA, MLH1, MRE11A, MSH2, OGG1, p53, RAD51 and RAD52 genes which are all involved in the DNA repair pathway. The prevalence of the 11 SNPs was compared between cases and controls. Genotype frequencies were compared across groups using the chi square test and the Mantel-Haenzel test for linear trend. The association between breast cancer and each SNP was examined using logistic regression with the SNP genotype tested under models of complete dominance and recessive inheritance. Three SNPs showed significant associations with breast cancer. For the most significant SNPs, the estimated ORs were 0.74 (95%CI 0.59-0.93) and 1.41 (95%CI 1.08-1.83) under a dominant inheritance model, with a combined Ptrend 0.0087 and 0.0076 respectively. These results suggest that a proportion of the SNPs under study are modifying breast cancer risk. Large numbers of samples will be needed to verify our results in other populations. We are currently expanding our analysis to include a greater number of SNPs and to evaluate potential underlying gene-gene or gene-environment interactions, in order to advance our knowledge on the effect of genetic polymorphisms on breast cancer susceptibility in Cypriot women.

#### P07.021

#### Variants in the vitamin D receptor gene and breast cancer

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Breast cancer is the most commonly occurring cancer among women, constituting 23% of all cancers. 5-10% of all breast cancers are caused by germ-line mutations in *BRCA1* and *BRCA2*. Multiple low-risk genes with variants common in the general population are thought to produce a mild susceptibility risk to sporadic breast cancer.

The vitamin D receptor (VDR) gene is a key mediator in the vitamin D pathway, and has been of long interest in breast cancer aetiology, since vitamin D exposure has been reported to reduce breast cancer risk. In the present study we have explored the implication of VDR in sporadic breast cancer, in the Spanish population since previous studies have been done in different populations than South European ones.

A total of 576 healthy controls from the Spanish population and 576 consecutive and non-related sporadic breast cancer cases, collected from different hospitals in Spain, were used in this study. Genotyping studies were carried out over four SNPs within the *VDR* gene, located on exons, in the putative promoter region or in untranslated regions. Genotyping was performed using TaqMan.

We detected associations for two of the selected SNPs: rs10735810 with OR=1.49 (95% C.I. 1.01-2.21; p=0.045), and rs731236 with OR=0.72 (95% C.I. 0.51-1.02; p=0.064). We also studied both haplotype and diplotype using PHASE v2.0, and detected associations with disease that were considered with the genotype results. Additionally, VDR proliferation parameters such as tumor differentiation grade and tumor aggressiveness will be discussed.

#### P07.022

#### Mutations in CARD15 and smoking confer susceptibility to Crohn's disease in the Danish population

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INTRODUCTION: Three mutations in the CAspase Recruitment Domain gene (CARD15) predispose to Crohn's disease (CD) in Caucasian populations.

The frequencies of the three most common CARD15 mutations differ greatly between ethnic groups. Heterogeneity even exists between the European countries.

AIMS & METHODS: The aim of this study was to investigate the mutation frequency in patients with inflammatory bowel disease and healthy controls in Denmark. Genotyping of the three CARD15 mutations were performed in 388 patients with Crohn's disease, 565 patients with ulcerative colitis and 796 healthy controls using Real-Time PCR. A comparison of allele and genotype frequencies in the three groups was made. A possible additive effect of smoking on CARD15 mutations was also examined.

RESULTS: CARD15 mutations were significantly more common in CD patients compared with healthy controls (21% vs. 10%; P<0.001). A gene-dosage effect was observed ( $OR_{adj,smoking}$  22.2; P<0.001 for two CARD15 mutations vs.  $OR_{adj,smoking}$  1.8; P=0.01 for one CARD15 mutation). The 1007insC protein truncating mutation was the major contributing mutation. Among the Danish CD patients ileal involvement was more common among patients having CARD15 mutations ( $OR_{adj,smoking}$  3.6; P<0.001). Smoking was independently associated with CD (OR 1.8; P<0.001), and no additive effect of smoking on CARD15 genotypes was found.

CONCLUSION: In the Danish population CARD15 mutations were associated with Crohn's disease. The CARD15 mutation frequency was in agreement with the lower frequency found in Northern European countries. Smoking was found to be a risk factor for developing CD.

#### P07.023

#### Cultural practice of consanguineous marriages in Morocco : prospective study in Rabat city

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The practice of consanguineous marriages has been widespread for hundreds of years, and is still very common, especially among various Middle Eastern, Asian and African populations. Their health consequences are primarily linked to the increased risk of congenital malformations and autosomal recessive disease.

The present study set out to determine the frequency of consanguineous marriages, identify and analyze the factors associated (bivariate analysis) with a randomly selected sample of 270 mothers postpartum in maternity of the Souissi Hospital in Rabat city (north-west of Morocco), between November 2004 and June 2005. All information was based on structured face-to-face interviews.

The Results showed that 21% of marriages were consanguineous (95% CI 12-28%), among which 70% were between first cousins. According to these results, several variables are associated with the choice of this type of marriage, namely women's education, their age at first marriage, place of residence, as well as type of childhood residence (rural or urban).

#### P07.024

#### Matrimonial choice in the Souss Massa Draa region in Morocco

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The study of geographical endogamy has relevance for indications on the genetic variability of the population, given that related parents have more chances to carry the same alleles, favouring homozygosity in their children.

The objective of this study was to evaluate geographical endogamy in the population of the region of Souss Massa Draa in south Morocco, as an indicator of the degree of genetic isolation of the population.

A prospective study was carried out between October 2005 and April 2007 on 190 randomly picked families of Souss Massa Draa. The choice of the mate was evaluated according to the geographical origin

of the couple and their parents. The intensity of the endogamic behaviour is determined by the endogamy ratio.

The results indicated clearly a tendency of the couples to geographical endogamy. 95% of the couples involved in the study, chose a mate of the same geographical origin, 98% did so in the generation of their parents.

The intergenerational comparison of the measured endogamy ratio shows the same degree of genetic isolation for both generations ( $p>0.05$ ).

Compared to other results obtained in other regions of Morocco, the Souss Massa Draa region can be qualified as "closed", which reflects the cultural and traditional heritage of this region.

#### P07.025

##### The frequency of cholesteryl ester transfer protein TaqIB polymorphism in the Western Anatolia

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The main aim of our studies is to indicate Taq1B polymorphisms in Cholesteryl Ester Transfer Protein (CETP) gene in healthy Turkish population as statistically and to explore the difference between other populations. We have examined allele frequencies for cholesteryl ester transfer protein (CETP) gene Taq1B polymorphism in our 100 healthy control subjects (74 men and 26 women) residing in Izmir (Turkey), by PCR-RFLP. We found the ratio for B1/B1 carriers as 30.0%, for B1/B2 carriers as 45.0% and for B2/B2 carriers as 25.0%. B1 allele frequency was 52.5% and B2 allele frequency was 47.5%. Depending on this results we observed that percentage of homozygotes for the B1 allele is lower in our samples than other populations (Spain, Greece, Japan, France, Scotland, Finland, USA) but for the B2 allele, it is much more higher than the other populations which have been studied before in other countries. The percentage of heterozygotes in our population is also mostly lower than the other populations except healthy Spanish population.

#### P07.026

##### Long-term cytogenetic effects of natural radiation on Gudalore inhabitants in India

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Some locations in the Indian subcontinent have high levels of natural radiation due to the presence of monazite along with other heavy minerals such as ilmenite, zircon, garnet, etc. Gudalore situated on the Nilgiri Mountain, is the oldest and important ecosystem in peninsular India. Indoor gamma radiation dose rates were measured inside residential buildings in Gudalore using a CaSO<sub>4</sub>:Dy thermoluminescent dosimeter for 1 year. The dose rates observed are between 77.9 and 229.3 nGy h<sup>-1</sup> and may be attributed to the type of building materials used in the dwellings monitored. The pioneering work on cytogenetics has evolved a long way for detection of various clastogenic insults and biodosimetry can help to verify the result derived from physical dosimeters during latter's improper use.

The focal aim of the present investigation was to identify the chromosomal aberrations in Gudalore area. In order to investigate the possible cytogenetic damage to the residents of contaminated buildings, a G-banding method was carried out on the lymphocytes of 61 radiation-exposed individuals and equal number of controls selected. Experimental and controls were selected by confirmed by TLD measurement. In the present study volunteers provided blood samples (5 ml) to establish cell cultures at 52 h. For karyotyping, 40 complete metaphase cells from each subject were evaluated.

All the recognizable structural aberrations of chromosomes or chromatid were recorded and statistically analyzed. In 3 experimental cases showed dicentric chromosomes. In addition, the chromosomal regions close to the centromere were found to break more frequently than elsewhere in the genome.

#### P07.027

##### ABCC1 polymorphisms contribute to level and decline of lung function in two general population cohorts

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**Background:** The metabolism of xenobiotics plays an important role in smoking related lung function loss and the development of Chronic Obstructive Pulmonary Disease (COPD). The *ATP-Binding Cassette, Subfamily C, Member 1* (ABCC1 i.e. Multidrug Resistance Protein 1) is a membrane pump excreting a variety of xenobiotics from the cell. We previously showed a lower expression of ABCC1 in bronchial epithelium of COPD patients than of healthy controls, and even further decrements in more severe COPD stages.

**Methods:** We tagged ABCC1 using all 51 prevalent (minor allele frequency >5%) and non-correlated ( $r^2<0.8$ ) single nucleotide polymorphisms (SNPs). Effects of SNPs in relation to the level and longitudinal course of lung function were assessed in two independent, prospective, population-based studies, i.e. the Doetinchem (n=1,152) and Vlagtwedde-Vlaardingen (n=1,390) cohorts.

**Results:** Two SNPs (rs212093 and rs4148382) in the 3' untranslated region of ABCC1 were significantly associated with a lower Forced Expiratory Volume in one second (FEV<sub>1</sub>) in both cohorts. Four moderately correlated ( $0.5 < r^2 < 0.8$ ) SNPs (rs4148330, rs4781699, rs8045000, and rs7190484) in the ABCC1 promoter and intron 1 had a significant effect in both cohorts of the same, negative, magnitude, but on different lung function outcomes, i.e. FEV<sub>1</sub> or FEV<sub>1</sub>/VC (Vital Capacity) ratio. One SNP in intron 14 (rs35621) was significantly associated with a highly excessive FEV<sub>1</sub> decline in both cohorts.

**Conclusions:** This is the first study showing a significant relation between ABCC1 SNPs and lung function level and decline in two independent population-based cohorts. These SNPs are therefore putative candidates for studies to prevent COPD development and pharmacogenetics in established COPD.

#### P07.028

##### The relation of common diseases SNPs and life span: no evidence for shared genetic component

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Life span is a multifactorial trait with a strong genetic component. Besides the number of genes has been directly implicated into the processes of ageing and longevity, the genes responsible for common diseases such as cardiovascular (CVD) or immunity disorders are also had to be under intent consideration. The persistence and both elimination from human populations 'predisposing' common genetic variants is forced by a complex interplay of different evolutionary processes; some variants being unfavorable during particular periods of life can offer their owners advantages on the others. Latter should be taken into account e.g. while developing genetic test and interpreting frequently discrepant genetic data on disease association. Testing the hypothesis that CVD predisposing alleles can be related to the life span, we investigate several cohorts: healthy controls of childbearing age (aged from 20 to 45, n=282), nonagenarians (aged 90 and over, n=235) and patients with cardiovascular disease (arterial hypertension with different complications) (n=231) from the same geographic region and ethnicity (East Siberia, Russians) and genotyped for several well-known CVD candidate genes polymorphisms (G-308A TNF, C894T NOS3, and A1166C AGTR1). While all the three SNPs under study affected several important cardiovascular endophenotypes (arterial blood pressure measurements, cardiac parameters, left ventricular hypertrophy development est.) no significant differences had been revealed among nonagenarians and middle aged group. In this study we never found actual proof for the stated hypothesis, though a huge

amount of other genetic variants and the larger samples are needed to be tested before its decline.

#### P07.029

#### Natural Background Radiation Dictates Extensive Structural Polymorphism in the Human Y-Chromosome

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Ionizing radiations are known to affect the human genome but its impact on human Y chromosome still remains unaddressed. Here we analyze 300 males residing in an area containing the world's highest level of natural background radiation (NBR) and 390 controls from different parts of India. Random microdeletions were observed in 95% of the NBR exposed males with higher frequency in AZFc region but without gr/gr or b2/b4 phenotypes. Scrutiny of the male fertility associated genes showed copy number polymorphism (CNP) and tandem duplication in 84%, and exclusive deletion of *DBY* in 25% of the exposed males. Detailed analysis revealed multiple polymorphic copies of *SRY* and *CDY1* genes in addition to several known and novel mutations of the *SRY* gene. Amongst NBR exposed males with multiples copies of the *DAZ* genes, 75% showed varying FISH signals for *DAZ* genes with unilocus or bilocus duplications whereas 30% showed mosaicism in terms of presence/absence of the signals in 6-8% cells and unexpected number of signals in 9-12% interphase nuclei. Interestingly, all these alterations were exclusively somatic in nature substantiating normal fertility status of NBR exposed males. Though the actual mechanism is not known, we hypothesize that some putative innate protective mechanisms are operative in germline to counteract the effect of NBR. Analysis of additional Y linked loci may uncover the overall impact of NBR on the structural and functional attributes of this chromosome.

#### P07.030

#### Genome-wide screen for copy number variation in a Finnish schizophrenia case-control sample identifies a submicroscopic deletion in 22q

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Copy number variations (CNV) are likely to comprise considerable source for diversity in human genome and in determining heritable susceptibility to diseases with complex etiology. We utilized Genome-wide SNP-array data from Illumina 318K genotyping platform on a Finnish sample of 200 familial schizophrenia cases and 200 controls with well established genealogy to search for CNVs potentially predisposing to schizophrenia. Half (47%) of the sample originated from an Internal Isolate of Finland with an exceptionally high prevalence of schizophrenia (3% versus 1.2% for the general population). The sample was a part of a large international EU-funded consortium, SGENE, combining 1,454 schizophrenia patients and 1,600 controls of European origin. In the Finnish sample we identified a novel 240-280 kb heterozygous deletion in chromosome 22q11.22 in 13 patients and three controls all originating from the Isolate. We extended our investigations to 17,808 additional European population controls (2,525 from Finland) and 1,254 cases of European origin including the rest of the SGENE project. Among these individuals we found 29 carrying the deletion, 22 of which originated from the Isolate. In a Finnish schizophrenia family sample we found 27/354 affected family members and 51/736 unaffected family members carrying the deletion. One mentally retarded individual was homozygous for the deletion. The deletion overlaps with three genes, *PPM1F*, *TOP3B* and *IGLV2-14*, not previously linked to schizophrenia. Our finding confirms the impact of population bottleneck on the enrichment of large CNV and implies the impact of this 22q

region in the etiopathogenesis of familial schizophrenia.

#### P07.031

#### Analysis of normal human variation to identify mechanisms of the monogenic disease, cystinosis

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Mutations in *CTNS* on 17p13 cause cystinosis, a rare autosomal recessive disease characterized by defective transport of cystine out of lysosomes. Using complex genomic approaches we studied the biology of *CTNS* in large unaffected families assuming normal human variation in *CTNS* expression could inform us about this gene's function. Using lymphocyte samples from 1240 individuals, we significantly detected over 20,000 transcripts, including *CTNS*. To identify regulatory variants, we resequenced *CTNS* in 191 founders, identifying over 180 SNPs for genotyping. Analysis of these variants with *CTNS* expression revealed strong evidence for *cis*-regulation (*p*-values as low as  $2.4 \times 10^{-39}$ ). We performed association analysis on the transcriptional profiles to identify genes causally downstream of *CTNS*. Preliminary analyses revealed multiple genes related to the mediation of polyglutamine tracts and the unfolded protein response. To identify *trans*-acting upstream genes influencing *CTNS* expression, we performed both genome-wide linkage and association scans. Genome-wide linkage results identified a region at 9q21 (*p* = 0.0025). Examination of the genetic correlations of expression levels of genes within this region with *CTNS* expression identified a novel candidate gene, *VPS13A* (vacuolar protein sorting 13A). *VPS13A* and *CTNS* expression levels were inversely genetically correlated (*p* =  $9 \times 10^{-6}$ ), indicating *VPS13A* as a potential inhibitor of *CTNS* expression. Sequence variation in *VPS13A* also influenced *CTNS* expression (*p* = 0.009). Genome-wide association results identified five additional *trans*-acting regions containing several reasonable positional candidates. These results show the biological value of integrative genomic and transcriptomic approaches for the identification of disease gene function using normal human variation.

#### P07.032

#### CYP2D6 genetic polymorphism in Mexican Nahuas

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*CYP2D6* catalyzes the metabolism of 25-33% clinically important drugs and is highly polymorphic. There are at least 50 different alleles of *CYP2D6*, which are correlated to ultrarapid, extensive and poor metabolizer phenotypes. Interethnic differences in allele frequencies of *CYP2D6* have been documented for Europeans, Asians, Africans and Hispanics; however data on Amerindians are scanty and limited to a few populations. Nahuas are individuals that speak Nahuatl and comprise the largest Mexican indigenous population. Nowadays, Nahuas can be found at Mexico D.F. Southern area (central group) and in other states (peripheral group). In this study 315 donors representing 7 different Nahuas populations were genotyped for *CYP2D6\*3*, *CYP2D6\*4*, and *CYP2D6\*10* by PCR-RFLP. Additionally, *CYP2D6\*17* was determined in the Coyolillo group as previous data revealed a predominant influence from African origin (Buentello-Malo, 2003). The frequency of *CYP2D6\*3*, 4, and 10 among 315 Nahuas was 1.6%, 12.2% y 1.9%, respectively; while *CYP2D6\*17* frequency was 32% in 36 Coyolillo Nahuas. Allelic frequencies for each group are presented in the table and compared to data from Mexican Mestizos (López, 2005). Our findings show that Nahuas groups are heterogeneous and different from Mexican Mestizos. This study will extent the knowledge of the genetic structure of Mexican Nahuas; in particular it could have important implications for the use of drugs that are substrates of *CYP2D6* and have a narrow therapeutic index.

CYP2D6 allele frequencies				
Group	N	CYP2D6*3	CYP2D6*4	CYP2D6*10
Zitlala, Gro.	44	0.0	7.4	0.0
Chila, Gro.	27	1.8	9.2	0.0
Ixhuatlancillo, Ver.	47	0.0	1.1	2.1
Necoxtla, Ver.	36	9.7	20.8	2.8
Coyolillo, Ver.	36	1.4	16.7	5.6
Milpa Alta, D.F.	23	2.2	6.5	0.0
Santo Domingo, Mor.	102	0.0	16.0	0.2
Mexican mestizos	243	1.44	11.21	12.45

**P07.033****Frequency of CFTR mutations in infertile Italian individuals**

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In our institute, one of the main Italian private healthcare institutions focused on out-patient and day hospital services, we perform the analysis of 57 mutations and of poly-T polymorphism in intron 8 of the Cystic Fibrosis gene (CFTR) on patients of Medically Assisted Procreation (MAP) Centers.

At the present, more than 1400 different mutations have been registered in the CF Mutation Database (<http://www.genet.sickkids.on.ca/cftr>); few of them show frequencies higher than 1% whereas most of them are "private mutations". Analysis of the Italian population show patterns of genetic differentiation: the "capture rate" of the test in use is quite different for various Italian regions, ranging from 74.7% for Lombardia to a maximum of 88.3% and 88.1% for Basilicata e Sardegna respectively (data inferred by the frequency of CFTR mutations in different Italian regions (1)). We present results about the analysis on 2320 individuals comparing them with those obtained by the authors in other MAP Centers (2): as expected, the frequencies of the analysed CFTR mutations show significant differences.

We do believe that data obtained in infertile individuals could be very useful for evaluation of carriers frequency in general population.

(1) The molecular genetic epidemiology of cystic fibrosis. Report of a joint meeting of WHO/ECFTN/ICF(M)A/ECFS, Genova, 19 Giugno 2002

**FREQUENZA DELLE MUTAZIONI CFTR IN COPPIE INFERTILI**

(2) Frequenza delle mutazioni CFTR in coppie infertili

A.Verri, M.Bologna, T.Sarno and A. Montanelli

38° Congresso Nazionale della Società Italiana di Biochimica Clinica e Biologia Molecolare Clinica, Torino, 19 - 22 settembre 2006

**P07.034****The analysis of polymorphism CYP1A1 and CYP1A2 genes at the population of Russia (Republic Bashkortostan)**

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In three ethnic groups of Republic Bashkortostan: Russian (N=451), Tatars (N=333) and Bashkirs (N=171) was studied distribution of alleles, genotypes and haplotypes polymorphic loci CYP1A1 (A2455G, Ö3801Ñ) and CYP1A2 (-2464T/delT, -163Ñ/Å). It was shown the similarity of groups Tatars and Russian on haplotypes frequencies CYP1A1 gene ( $\chi^2=0.97$ , df=3,  $\delta=1.00$ ) è CYP1A2 gene ( $\chi^2=1.55$ , df=3,  $\delta=0.92$ ). We found significant differences in haplotypes frequencies CYP1A1 gene between the ethnic group Bashkirs and groups Tatars and Russian ( $\chi^2=12.33$  df=3,  $\delta=0.008$ ;  $\chi^2=9.22$  df=3,  $\delta=0.034$ , accordingly), that is connected with high frequency of CYP1A1\*2B (10.17%) among the Bashkirs.

The Mspl locus (T3801C) is in linkage disequilibrium with the A2455G polymorphism in the Bashkir population, the Tatars and Russian (1.61%) populations. The frequency of the CYP1A2\*1D haplotypes was highest in the Bashkir population (11.02%) compared to frequencies that were found in the Tatars (2.36%) and Russian (1.61%) populations. Similar distinctions of on haplotypes frequencies CYP1A1 gene an ethnic group the Bashkir with Russian and Tatars ( $\chi^2=18.78$ , df=3,  $\delta=0.0001$  and  $\chi^2=14.33$ , df=3,  $\delta=0.003$ , accordingly). The CYP1A1\*2A haplotype was meet with High frequency in all ethnic groups. The frequency of CYP1A1\*2A haplotype considerably increased in The Bashkir ethnic group (15.79%) that is comparable to frequency of this haplotype in Asian populations (14.9%). The increasing of the CYP1A1\*2A haplotype frequency in the Russian ethnic group up to 9.0% in comparison with 5.8% in samples of Europeans also reflects presence Asian components in origin of Russian ethnic group living in

Republic Bashkortostan.

**P07.035****A study of the Insertion-Deletion polymorphism of the gene CYP2E1**

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INTRODUCTION: We studies gene, encoding ferments of the first phase biotransformation xenobiotics, cytochrome P-450 2E1(CYP2E1). CYP2E1 coding the ferment N,N-dimethylnitrosoamin-n- dimethylazyl is located on the chromosome 10 (q24.3) consist of 9 exons and 8 introns.. In given gene exists polymorphism, given by insertion 96 p.b. in promoter region (the allele: \*D - 633 p.b., \*I - 729 p.b.). The ferment, coded gene, have charge of removing ethanol and acetone expression level is significantly lower in other organs and tissues, in particular, kidneys, pancreas, brain, lung, nasal and intestinal mucosa.

Materials and methods: We studied 3 groups: individuals living in region with disadvantage ecological condition, group persons concerning with sport, and the general sample. The individuals age made up from 14 up to the ages of 30. The analysis genetic polymorphism is realized by polymerase chain reaction (PCR).

RESULTS: In group of the inhabitants region with disadvantage ecological condition frequency genotype \*D/\*D vastly increased (96.2%), but in group athlete tends to reduction (58.5%) in contrast with the total sample (69.2%). The frequency genotype \*I/\*D is lowered in group of the inhabitants region with disadvantage ecological condition (3.8%) and increased beside athlete (41.5%) in contrast with the total sample (30.4%). In sharing the frequencies alleles in group of the inhabitants region significant increasing of the frequency allele exists with disadvantage ecological background \*D (98.1%) and reduction allele \*I (1.9%) in contrast with the total sample (84.4% and 15.6% accordingly).

**P07.036****The effect of the DAOA (G72/G30) gene and developmental risk factors on depression and related temperament traits in Finnish birth cohort**E. S. Nyman<sup>1</sup>, P. Soronen<sup>1</sup>, J. Miettunen<sup>2,3</sup>, A. Loukola<sup>1,4</sup>, M. Joukamaa<sup>5,6</sup>, P. Mäki<sup>2</sup>, M. Järvelin<sup>7,8</sup>, N. Freimer<sup>9</sup>, L. Peltonen<sup>1,10</sup>, J. Veijola<sup>2,3</sup>, T. Paunio<sup>1,11</sup>;<sup>1</sup>FIMM, Institute for Molecular Medicine Finland and National Public Health Institute, Helsinki, Finland, <sup>2</sup>Department of Psychiatry, University of Oulu and Oulu University Hospital, Oulu, Finland, <sup>3</sup>Academy of Finland, Helsinki, Finland,<sup>4</sup>Finnish Genome Center, University of Helsinki, Helsinki, Finland, <sup>5</sup>Tampere School of Public health, University of Tampere, Tampere, Finland, <sup>6</sup>Department of Psychiatry, Tampere University Hospital, Tampere, Finland, <sup>7</sup>Department of Epidemiology and Public Health, Imperial College, London, United Kingdom, <sup>8</sup>Institute of Health Sciences, University of Oulu, Oulu, Finland, <sup>9</sup>Semel Institute for Neuroscience and Human Behavior, University of California, Los Angeles, CA, United States, <sup>10</sup>Wellcome Trust Sanger Institute, Cambridge, United Kingdom, <sup>11</sup>Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland.

D-Amino acid oxidase activator (DAOA or G72/G30) encodes for a protein with a possible role in glutamate signaling, and it has been implied in the development of schizophrenia and bipolar disorder. It has been suggested to increase susceptibility to episodes of mood disturbances across diagnostic boundaries. We investigated the role of DAOA in depression in a population-based Northern Finland Birth Cohort 1966 (n=12058). We ascertained a sample based on developmental risk factors (high risk n=285, low risk n=926) with a clinically postulated connection to depression susceptibility, including difficulties during the perinatal or early childhood periods or related to the psychosocial environment, to dissect genetic effects increasing liability to depression from environmental effects. Depressive symptoms were quantified according to the Symptom Checklist (SCL) and dichotomous depression was classified as self-reported depressive disorder diagnosed by a clinician. We also wanted to examine Cloninger's Temperament and Character Inventory (TCI) temperament traits because of previous affirmative reports, especially on Harm avoidance to depressive disorder. We genotyped evenly spaced haplotype tagging SNPs at the DAOA locus, and performed linear regression and case/control association testing against the selected traits. In preliminary analyses we detected an association of DAOA with depression in low risk females and with depressive symptoms in low risk males. We

also found suggestive association of DAOA with the Harm Avoidance subcomponent 2 (HA2), Fear of Uncertainty, in high risk males and females. Our findings support the role of DAOA in depression and in a related temperament trait, HA2.

#### P07.037

#### **DFNB1 mutations outside the GJB2 coding region in UK hearing loss referrals: testing and prevalence**

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GJB2, a component of the recessive *DFNB1* locus at 13q, encodes connexin 26 and is the major gene in non-syndromic sensorineural deafness. Pathogenic *DFNB1* mutations that map outside of the *GJB2* coding region exist, but routine clinical testing often focuses on the single coding exon of *GJB2*. This bias presents as a high proportion of unresolved subjects with single *GJB2* coding region variants relative to the background carrier frequency. Untested or uncharacterised *DFNB1* alterations are likely to account for the excess. We investigated the significance of two well-documented *DFNB1* changes, the large deletion g.(GJB6\_D13S1830)del and the splice site mutation c.-23+1G>A (also known as IVS1+1G>A or 1-3170G>A) of the non-coding exon of *GJB2*, in a large mixed stream of hearing loss referrals from the UK. Our set comprised 1425 apparently unrelated index subjects with a 80:20 ratio of Caucasians to non-Caucasians (predominantly British Asians). We developed and validated fluorescent assays for medium-throughput typing and analysed, prospectively and retrospectively, the vast majority of appropriate cases. In our Caucasian subgroup, the contribution of g.(GJB6\_D13S1830)del to the detectable *DFNB1* mutation spectrum is 5.5% (95% CI: 2.9% - 9.4%) while the relative frequency of c.-23+1G>A is 1.4% (95% CI: 0.3% - 4.0%). g.(GJB6\_D13S1830)del is absent in our non-Caucasian subgroup, but c.-23+1G>A is detected in around 10% of all pathological genotypes, including homozygous occurrence in consanguineous pedigrees. Concentrating on the c.-23+1G>A mutation we discuss genotype-phenotype correlations, impact on *GJB2* expression, and evolutionary origins.

#### P07.038

#### **The investigation of dermatoglyphic samples in patients with Parkinson**

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The irregularities in the number and the shape of chromosomes destroy dermatoglyphic samples expressed by minor-effective genes and helping diagnosis of the hereditary diseases.

The most valid idea for the reason of Parkinson is that, both genetic and environmental factors associate in the formation of the disease.

The aim of this study is searching the relation between Parkinson and dermatoglyphics. Due to this, dermatoglyphic samples of 55 cases with Parkinson were evaluated by comparing the samples of the control group including 48 healthy people.

Investigating samples from the patient and the control group by "Rontgen Film method, an increase in the number of the knots and a decrease in the number of the loops at the I. finger of the right hand; a decrease in the number of triradiiases at the left hand interdigital regions I and III and the right hand interdigital region I; decrease in the number of triradiiases located on the right and the left hand tenar region and on the right hand hipotenar region; a decrease in the number of right hand a-b lines were detected. In our study, significant differences were found in the finger tip and palm samples of the Parkinson patients ( $p<0.05$ ). Even though data were related to a limited number of cases, they were found to be interesting due to giving a clue to the genetic predisposition of Parkinson. Being easy to perform, fast and cheap, not causing trauma, dermatoglyphic analyse methods suggest taking place in the scanning tests of Parkinson patients.

#### P07.039

#### **The Accumulated Number of Risk Variants (=Risk Value) Rather Than Anyone of Them Determines Risk of Diabetes Mellitus Type II**

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We determined the distribution of selected SNPs along inflammatory and metabolic pathways among Diabetic (DM2) patients from two different Mediterranean populations, one Southern European (Malta; N = 200) and one North African (Libya; N = 200). The reference populations were pools of DNA from consecutive newborn from the Malta BioBank (N = 200) and from Tripoli (N = 200) respectively. Significant differences between patients and reference groups in both populations have been found in ADRAB2 (51.1% vs 23.4%) FABP2 (32% vs 8.6%) UCP1 (26.4% vs 14.1%) and LEPTIN (35.8% vs 27.3%). Odds ratios were determined with respect to the inheritance of Sniplotypes, being defined as the non-random association of the 5 more significant non-syntenic genetic markers. The frequency of the wild sniplotype "1.1.1.1." (where 1 = wild type homozygote, 2 = heterozygote and 3 = mutant homozygote) and Leptin heterozygotes (1.1.1.2.1) were 1:10 compared to the reference populations. Additional statistical comparisons were confounded by the small number of cases falling in many categories. However, the distribution of relative risk / sniplotype suggested that the sniplotypes with increased 2s or 3s carried increased risk. In fact, it could be shown that relative risk increased exponentially with the number of inherited variant alleles ( $R^2 = 0.97$ ; N = 800). Thus, it can be concluded that the probability or genetic risk of developing type II Diabetes is determined by the number of variant alleles rather than the pathophysiological impact of any one of them.

#### P07.040

#### **Biological effect monitoring in cigarette smokers from the Kudankulam nuclear power project site area exposed to radiation, India**

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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. The focal aim of the present investigation was to identify the chromosomal alterations in cigarette smokers from Kudankulam nuclear power project site (KNPPS) areas located near to Kanyakumari. The levels of primordial radionuclides in Kanyakumari, a High Background Radiation Area (HBRA) exceeds 4.7 times than the normal (1 mSv).

In the present investigation, 72 smokers were selected in KNPPS area and equal numbers of smokers were selected in the normal level radiation area and it was used as a controls. After signing a consent form, volunteers provided blood samples (5 ml) to establish cell cultures at 52 h. For karyotyping, minimum 40 complete metaphase cells from each subject were evaluated.

Chromosomal and chromatid-types aberrations were observed in experimental subjects and controls. Some Dicentric chromosomes(DC) were particularly observed in experimental subjects. DC which are easy to identify also with classic Giemsa staining, represent an attractive biomarker, especially for assessment of radiation exposure. Statistical significant results were observed between experimental and control groups confirmed by Mann-Whitney U.

Information on the induction of radiation effects can be considered in the context of cancer risk by exposure to the radiation, this study gives potentially important information on the general health effects due to radiation exposure and the effect of cigarette smokers; it also helps people to understand the hazardous nature of chronic radiation exposure.

**P07.041****Comparative study about digit ratio in two human populations of Bihor county**

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In 1892 Sir Francis Galton published his classic treatises on fingerprints. Specifically, it is the ratio of the length of the index finger (digit 2, or "2D") and the ring finger (digit 4, or "4D") that is sexually dimorphic. We investigated 200 individuals. We measured the length of the 2nd, 3rd and 4th fingers of 100 from each locality (Oradea and Vascau). The Oradea locality has over two thousands of inhabitants, which means the variability of some phenotypical features must be a large one. The Vascau locality has not so many inhabitants, fifty thousand approximative. The individuals of Vascau were at random choosed and from Oradea are students. We measured the length of the fingers, from the finger basis to the superior bound of the phalanx. Then we calculated the digit ratio: 2D:4D, 2D:3D and 3D:4D. Also, we calculated z score and F distribution.

We compared the 2D:4D digit ratio of the studied localities ( $F = 7.39$ , z score =2.95). So, we may say the values proceed from two very different groups. So, the two studied groups are very different, the 2D:3D digit ratio of the two localities ( $F=1.217$ , z score=1.24) and the 3D:4D digit ratio of the two studied localities ( $F=2.06$ , z score=1.73).

We may conclude the two studied groups are very different. The variation coefficient demonstrated a very small variation of the values of 2D:4D, 2D:3D and 3D:4D values digit ratio in the each of the two studied populations.

**P07.042****Variants in DISC1 are associated with psychosis related traits in a large birth cohort**

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DISC1 is among the most studied susceptibility genes to schizophrenia and other major mental illnesses. Based on the genetic studies performed it seems that the risk conferred by the variants of DISC1 is small even in the highly selected populations. We wanted to study the effect of previously identified risk variants of DISC1 on quantitative endophenotypes for psychosis in an unselected population sample. We utilized the large birth cohort collected in Northern Finland (NFBC66). This study included 4444 individuals born in the area during the year 1966. We genotyped 41 SNPs covering DISC1, both upstream and downstream of the gene. Principally, we tested an a priori hypothesis of the interplay between three SNPs, rs1538979, rs821577 and rs821633 that had previously been reported to affect risk to schizophrenia and bipolar disorder. The test variables were four psychometric instruments selected to function as proxies for both positive and negative aspects of schizophrenia. These were Revised Social Anhedonia Scale (SAS), Revised Physical Anhedonia Scale (PAS), Perceptual Aberration Scale and Golden and Meehl Schizoidia scale. The results strongly support an effect of SNP rs821577 on SAS ( $P = 0.000021$ ) and PAS ( $P = 0.021$ ). Most importantly, certain combinations of the genotypes of the three SNPs that modify the risk of schizophrenia dependent on the local genetic background in a previous study also correspondingly modify the measures of SAS (best  $P = 0.0000022$ ) and PAS (best  $P = 0.0075$ ) in the birth cohort studied here.

**P07.043****Population study at STR loci Penta D and Penta E in Croatian population**

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In addition to our previous population studies of Croatian human population at 15 STR loci included in the AmpFISTR® Identifiler® system we have also analyzed the allele frequency distribution at two additional STR loci Penta D and Penta E in the representative sample of Croatian population since these highly polymorphic pentanucleotide loci included in the PowerPlex® 16 enhance the discrimination power of that system, which is important in resolving paternity disputes, and are also ideal loci for evaluation of DNA mixtures often encountered in forensic casework. A total of 200 unrelated Caucasian individuals born in Croatia have been sampled for the analysis. Buccal swabs have been used as the DNA source. The QuiAmp DNA blood mini kit was used for DNA extraction. The PowerPlex® 16 System has been used to simultaneously amplify 15 STR loci: Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Penta D, CSF1PO, D16S539, D7S820, D13S317, D5S818 and Amelogenin. STR loci were amplified in ABI GeneAmp® PCR Thermal Cycler according to the manufacturer's recommendations. Electrophoresis of the amplified products was preformed on an ABI 3130 genetic analyzer. Raw data have been compiled, analyzed and numerical allele designations of the profiles were obtained by using the accessory software: ABI 3130 Genetic Analyzer Data Collection v3.0 and GeneMapper™ ID Software v3.1. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated. We have also compared our data with data obtained from geographically neighboring European populations.

**P07.044****Linkage disequilibrium and distribution of the variable number of tandem repeats (VNTR) polymorphism and 1342 A/G polymorphism in exon 9 of the dopamine transporter gene (DAT1) in populations of Northern Eurasia**

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The DAT terminates dopaminergic neurotransmission and thus plays the important role in dopamine metabolism. Gene encoding the DAT (DAT1, SLC6A3) localized in the end of p-shoulder 5 chromosome. We've investigated two polymorphisms of this gene (VNTR in 3'-untranslated region and 1342 A/G polymorphism in exon 9) in seven populations: Buryat (n=105), Dungan (n=44), Persian (n=36), Kyrgyz (n=188), Tadjik (n=39), Ukrainian (n=94) and Uzbek (n=50). The observed genotype frequencies correspond to expected in Hardy-Weinberg equilibrium for both loci of all populations, excluding Tadjik population in exon 9-polymorphism. The alleles spectrum in VNTR polymorphism counted 6 alleles (from six tandem repeats to twelve), with 10 tandem repeats allele as prevalent in all groups (average frequency=0.834, variation from 0.707 in Ukrainians to 0.932 in Dungans). The prevalent allele in exon 9 was A allele (average frequency=0.817). The largest expected heterozygosity in both loci was founded in Ukrainian and Persian populations (~0.4). The high interethnic differences were found by maximum-likelihood chi-square statistics: most populations significantly differ from each other for both loci. The total level of genetic differences measured by Fst statistics was 3.2% (4.54 for VNTR and 1.86% for 1342 A/G polymorphism).

The normalized linkage disequilibrium coefficient was fairly strong ( $D'>0.6$ ) for all population, exclude Ukrainian ( $D'=0.3725$ ), Persian ( $D'=0.3759$ ) and Tadjik ( $D'=0.4212$ ). This data may be explained by different level of isolation during the ethnic history of Caucasoid (Persian, Tadjik, Ukrainian) and Mongoloid (Kyrgyz, Uzbek, Buryat, Dungan) populations. This work was supported by the Russian Foundation for Basic Research (project no. 06-04-48274-à, 07-04-01629-à).

**P07.045****Investigation of an effect of economic crisis on the prevalence of Down syndrome in St. Petersburg, Russia**N. V. Kovaleva<sup>1</sup>, D. K. Verlinskaya<sup>2</sup>, J. K. Morris<sup>3</sup>;

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**OBJECTIVE:** To investigate changes in the prevalence of Down syndrome (DS) births during a global social transition. **DESIGN:** Data from St. Petersburg Down Syndrome Register were analyzed with a special attention to the period of high social expectations (1983-87) when the number of births had been increasing (69,406→73,275) and the period of a social frustration (1995-99) when the number of births had declined most dramatically (33,841→29,438). **STUDY POPULATION:** During 1980-1999, 1358 liveborn children with DS were identified among 1 046,932 births; a rate of 1.3/1000 births. Prenatal diagnosis had low impact on the birth prevalence. **METHOD:** The data obtained were compared with the expected rates and numbers based on the data from NDSCR, 1989-2002. **RESULTS:** Maternal age distribution in the general population had changed during the study period: proportion of mothers aged ≥ 35 yr increased from 6.8% in 1983-87 to 8.4% in 1995-99, however this has not resulted in corresponding increase in DS rate. In contrary, DS rate in 1995-99 was the lowest over the study period, being 1.18/1000. However, by the model applied, this figure does not differ significantly from the expected. The DS infant mortality rate had fallen from 39% in 1983-87 to 17% in 1995-99. **DISCUSSION:** There is no evidence for an effect of economic crisis accompanied with psychological stresses and with nutritional deficiency on chromosome segregation or on surviving of affected children. A suggestion that low DS rate might be explained by better welfare standards of reproducing fraction of the population is under the study.

**P07.046****Skeletal Muscle Gene ACTN3 and Physical Performance:**O. Kasimay<sup>1</sup>, D. Sevinc<sup>2</sup>, S. O. Iseri<sup>1</sup>, K. Ulucan<sup>3</sup>, M. Unal<sup>1</sup>, A. I. Guney<sup>4</sup>, H. Kurtel<sup>1</sup>;

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ACTN3 gene is responsible from the production of alpha-actinin-3 protein, which has force-generating capacity of muscle fibers, and which is restricted to fast fibers. Homozygosity for 577X in ACTN3 (R577XX) results in no production of α-actinin-3 protein. Recent studies show that elite sprint athletes had a higher frequency of the RR genotype. Aim: The purpose of the study was to investigate ACTN3 gene variations and their probable phenotypic reflection by using physiological methods, and to show ACTN3 polymorphism in Turkish soccer players (n=31). Methods: After determining the genotypes by analyzing the blood samples, three groups (XX,RR,RX) were formed. The groups were existing R577X variant in both ACTN3 genes (XX,n=4), not existing R577X variant in both ACTN3 genes (RR,n=22), or existing R577X variant only one of the two ACTN3 genes (RX,n=5), respectively. To determine aerobic performance, Bruce protocol was applied on treadmill and maximal oxygen consumption (VO2max) was measured by metabolic analyzer. On a separate day, anaerobic performance was evaluated by Wingate test. Student's *t*-test or analysis of variance (ANOVA) was used for comparisons. Results: Thirteen % of the soccer players had homozygosity for R577XX codon. The VO2max levels in XX group tended to be higher from RR (*p*=0.09) and RX groups (*p*=0.05). VO2/HR (pulse oxygen) and VEmax (maximum ventilation) levels were not different between groups. Peak power values tended to be higher in RR group from the other groups. Our results evaluated the effect of genotypic variations on sprint and endurance performance of athletes contributing the understanding of genotype-phenotype correlation.

**P07.047****Polymorphisms of IL4 and IL4RA genes are associated with endometriosis**

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Endometriosis is defined as a condition which displays features similar to malignancy, proliferation and growth. Typical endometrioid cells are characterized by increased cytokine activity. Cytokine genes are polymorphic that results in synthesis of proteins with various functional activities. The goal of the study focuses on the role of allelic variants of IL4 and IL4RA genes in pathogenesis of endometriosis.

DNA samples from the patients with endometriosis (n=82) and healthy women without gynecologic complications (n=69) were included in the study. Group of patients was divided into two subgroups according to severity of disease. Polymorphisms of IL4 (-590T>C) and IL4RA (1902A>G) were defined by PCR-RFLP assay.

The alleles and genotypes frequencies of IL4 gene did not differ between studied groups (*p*>0.05). The frequency of G/G genotype for IL4RA gene was significantly higher in endometriosis patients (19.5%) as compared to the control group (1.4%, *p*<0.01). Also the frequency of allele G was significantly higher in patients (27.6% and 17.4%, *p*<0.01 respectively). The relative risk for endometriosis when having G/G genotype of IL4RA gene was estimated by an odds ratio of 16.51 (95% CI: 3.57-76.41). Also it has been shown that the frequency of C/C or C/T genotype of IL4 gene in the subgroup with severe endometriosis is significantly higher than in control group (*p*<0.05). The presence of allele C may be responsible for increased risk of endometriosis (OR=3.05; CI: 1.05-8.83).

Our data suggests that polymorphisms of IL4 (-590T>C) and IL4RA (1902A>G) may be treated as prognostic genetic markers of endometriosis.

**P07.048****Polymorphism C677T of the methylenetetrahydrofolate reductase gene and endothelial function in patients with essential hypertension of the Kazakh nationality**G. Junusbekova<sup>1</sup>, M. Tundybayeva<sup>1</sup>, G. Svyatova<sup>2</sup>;

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The purpose: to study association of polymorphic marker C677T of the methylenetetrahydrofolate reductase gene (MTHFR) with parameters of function of the endothelium in patients with essential hypertension (EH) of the Kazakh nationality.

Material and methods: A total of 118 patients (both males and females) of the Kazakh nationality were examined with EH of the II<sup>nd</sup> and III<sup>rd</sup> degrees average and high risk. We investigated endothelium - dependent vasodilation breakdown with reactive hyperemia by the method of D.Celermajer. The level of homocysteine (HC), endothelin-1, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and production of sE-selectin in blood was determined by immunohistochemical test with use of commercial kits. MTHFR 677C→T genotyping was detected by polymerase chain reaction.

Results: Distribution of genotypes of polymorphic marker C677T of the MTHFR gene in patients with EH appeared the following: C/C -45,8 %, C/T- 39,8 % and T/T-14,4 %. The frequency of the allele C was 65,7 %, and of the T allele it was 34,3 %. When comparing patients with genotype C/T and T/T (n=64) with those patients, having genotype C/C, we observed a smaller value of a gain of diameter of a brachial artery on a background reactive hyperemia (6,56±1,08 and 9,86±1,32 % accordingly, *p*=0,05). Besides in patients of the second group a higher level of sVCAM-1 (504,5±23,11 and 596±31,71 ng/ml accordingly, *p*=0,026) was observed.

Conclusions: In patients with EH in the Kazakh population the C677T polymorphism of the MTHFR gene can be associated with development of endothelial dysfunction.

**P07.049****Identification of point mutation pattern in the LDLR gene among Bulgarians with severe hypercholesterolemia**

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Familial hypercholesterolemia (FH) is a common, autosomal dominant disorder of lipid metabolism and is a major risk factor for coronary vascular disease. FH affects approximately 1 in 500 individuals worldwide. Mutations in LDLR, APOB and recently described PCSK9 genes have been associated with this disease. The aim of the present study is to provide information about the spectrum of point mutations in the LDLR gene in sample of 200 Bulgarian patients with severe hypercholesterolemia. We applied Single Strand Conformation Polymorphism (SSCP) analysis to screen for sequence variations in the coding regions and splice sites of the LDLR gene. The detected samples with different SSCP patterns were sequenced. In 29 cases the diagnosis FH was genetically confirmed. We found 7 point mutations (ex.4bG>A at 590, ex.9G>A at 1195, ex6C>A at 858, ex.8G>A at 1073 and T>C at 1061-8, ex.5A>C at 761, ex.11G>A at 1646) and 7 polymorphisms, one (ex.11C>T at 1705+23) not previously described. Our results have shown that exons 8, 9, 11 should be considered as "hot-spots" for Bulgarian population. In 3 out of 9 patients with determined mutations in the 11th exon we have observed in addition 2 different polymorphisms - C>T at 1705+23 and C>T at 1617. On the contrary, exons 16, 17, 18, 2, and the previously described as "hot-spots" exons 3 and 14 did not show aberrant profiles in the SSCP screening. Exons 7, 13 and 15 appear to be very polymorphic, however no mutations were observed in those exons.

**P07.050****Distribution of FMR1 alleles in two populations with different ethnic background: Madeira (Caucasian) and Guinea-Bissau (Sub-Saharan African)**

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The fragile X syndrome is the most common cause for inherited mental retardation. This syndrome results from an abnormal expansion of the CGG repeat in FMR1 gene which causes the inactivity of the gene, leading to the absence of the FMRP. The frequency of the alleles of FMR1 polymorphism was analyzed to the Madeira and Guinea-Bissau populations. Madeira and Guinea-Bissau population show significant differences ( $P<0.05$ ) but have some similar distributions, being the 29 repeats the most common allele followed by the 30 repeat CGG. These two alleles accounts for 51% of all alleles at this locus in a total of 19 different alleles varying between 7 and 38 repeats in the Madeira population and in the Guinea-Bissau population 22 different alleles varying in size from 8 to 43 repeats were identified and alleles 29 and 30 accounts for 45.2% of all alleles at this locus (table1). This population contains 89.6% of small alleles ( $\leq 35$  CGG) and 10.4% of large alleles ( $> 35$  CGG), a pattern similar to others observed in African populations but quite different from that of Caucasian populations. In the Madeira population ( $n=141$ ) no intermediate or permuted alleles were identified while in Guinea-Bissau population ( $n=104$ ) 4 intermediate alleles (prevalence: 1/26) were observed. These results can explain why there are no X-fragile reported patients in the Madeira population of 250.000 inhabitants.

(CGG)n	MA (N = 141)	GB (N = 104)
7	0.007	-
8	0.007	0.009
13	0.007	-
14	0.035	-
16	0.007	-
19	0.028	-
20	0.071	0.009
21	0.028	0.009
22	-	0.019
23	0.064	0.077
24	0.014	0.019
25	0.014	0.019
27	0.007	0.009
28	0.100	0.067
29	0.262	0.231
30	0.248	0.221
31	0.057	0.144
32	0.021	0.019
33	-	0.009
34	-	0.019
35	-	0.019
36	0.007	0.009
38	0.014	0.038
39	-	0.009
40	-	0.009
41	-	0.029
43	-	0.009
He	0.849	0.870

**P07.051****The genome-wide patterns of variation confirms significant substructure in a founder population**

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The genome-wide SNP genotyping platforms enable detailed association studies, but at the same time offer new insight into population genetics. Here we present an example of a founder population by scrutinizing nine geographically distinct Finnish subpopulations representing different eras in the population history to study the effect of bottlenecks and isolation using high-density SNP data. We demonstrate that population substructure and even individual ancestry are detectable at high resolution and support the concept of multiple historical bottlenecks resulting from founder effects.

We performed multidimensional scaling (MDS) of pairwise identity-by state (IBS) sharing data to delineate population structure. Within Finland the two primary dimensions of the MDS-analysis correspond remarkably with the east-west and north-south directions, respectively, showing a distribution of individuals corresponding closely with the geographical distribution of parents' birthplaces. The youngest subisolate showed higher IBS similarity compared to other subgroups and separation using an extremely fine resolution. We analyzed linkage disequilibrium (LD) and extended regions of homozygosity (ROHs) to further explore the genomic structure of the subpopulations. Highest LD and the largest number of long ( $> 10$  Mb) ROHs was identified in the youngest regional population and showed a gradual decline of these measures in older and more outbred, subpopulations.

The study shows the power of GWA data to trace the population history and also exemplifies the power to identify stratification even within homogeneous populations. A deeper insight into fine-scale population substructure also emphasizes the importance of adjustment of GWA studies aiming at identifying smaller and smaller genetic effects to avoid confounding.

**P07.052****Epidemiological study of Fraser syndrome in Europe**

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Fraser syndrome (FS) is rare autosomal recessive condition with classical features of cryptophthalmos, syndactyly, and genitourinary malformations. Abnormalities of the skull, ears, nose, and larynx are often present as well. Due to the rarity of FS, population-based epidemiological studies are lacking. We present the results of analysis of 24 cases of FS identified among 10 318 446 pregnancies registered in the EUROCAT network of congenital malformation registries in 1980-2004 period. This corresponds to a prevalence of 0.23/100000 or 1 in 429.935 births. Prenatal ultrasound examination detected abnormalities in 13/24 (54.2%) fetuses. Mean gestational age at discovery of an abnormality by prenatal ultrasound was 22.3±3.2 (18-27) gestational weeks. There were 2/24 (8.3%) fetal deaths, 9/24 (37.5%) pregnancy terminations and 13/24 (54.2%) were live born. One third of live births did not survive the first week of life. Male:female ratio was 3.4 (17/5). The mean birth weight in live births was 2363 ±622 g for males and 2133±413 g for females. The mean gestational age at birth was 37 weeks for both sexes. The most frequent associated congenital malformations were urogenital (81.8%; 18/22), eye (72.7%; 16/22), and limb (59.1%; 13/22) anomalies. The mean maternal age at birth was 28 ± 5 years and the mean paternal age 31±4 years. Parental consanguinity was present in 7/14 cases, and four families had already one affected child (4/13). All cases were registered in the Western part of Europe, 12/24 (50%) cases being from Great Britain and Portugal (prevalence 0.49/100000 or 1 in 202859 births).

#### P07.053

#### Analysis of frequencies of 35delG mutation in connexine 26 gene in different ethnic groups of Russia

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Mutations in the *GJB2* gene are a major cause of autosomal recessive and sporadic non-syndromic hearing loss in many populations. This study aims to determine the frequencies of 35delG mutation in different ethnic groups of Russia.

The patients with non-syndromic hereditary hearing loss from four ethnic groups (Chuvashs, Udmurths, Bashkirs and Russians from Rostov Regions) were analyzed for 35delG mutation in connexine 26 gene. Analysis of 60 patients with non-syndromic hereditary hearing loss from Chuvash Republic, 85 patients from Rostov Region, 36 patients from Republic of Bashkortostan, 58 patients from Udmurt Republic, showed that frequency for 35delG mutation was specific to Russian ethnic group (42.86%, 45%, 8.33%, 62.5%, respectively). Among the patients with non-syndromic hereditary hearing loss from other ethnic groups, frequencies of 35delG mutation were 5% for Chuvashs, 2.44% for Udmurths, 6.06% for Bashkirs.

More than 2574 healthy donors from five ethnic groups from Russia (Chuvashs, Maries, Udmurths, Bashkirs and Russians from Tver and Rostov Regions) were analyzed for 35delG mutation in *GJB2* gene. Significant differences in mutation frequencies between different ethnic groups were discovered. Analysis showed that frequency of 35delG mutation for Chuvashs ethnic group was 0.48% (5 chromosomes with mutation 35delG out of 1040 analysed (5/1040)), for Maries - 0.99% (8/804), for Udmurths - 0.25% (3/1184), for Bashkirs - 0.25% (2/792), for Russian - 1.44% (19/1320).

According to this research, contribution of 35delG mutation in connexine 26 gene to the development of non-syndromic hereditary hearing loss in various ethnic groups of Russia is different.

#### P07.054

#### N680S and -29 (G->A) FSH-R polymorphisms in Czech fertile male and female population

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The aim of this study was the determination of the genotype characteristics of FSH-R polymorphism in position -29 (G->A) and exon 10

N680S in fertile couples in families indicated for prenatal diagnosis, or with risk of cystic fibrosis, risk of trombophilic disorders and chronic pancreatitis disposition in male and female Czech population.

Polymorphism -29 (G->A) was examined in 318 females and 303 males, exon 10 N680S in 317 females and 304 males. The exon 10 and promotor polymorphisms were analyzed by allelic discrimination on ABI Prism 7000 detection system (Applied Biosystems).

The promotor polymorphism A/A in females was 5.03 %, in males 6.93 %; A/G in females 37.74 %, in males 35.31 %; G/G in females 57.23 %, in males 57.76 %. Exon 10 polymorphism Asn/Asn was 37.22 % in females, 27.96 % in males; Asn/Ser in 47.32 % for females, 50.33 % for males; Ser/Ser in 15.46 % in females and 21.71 % in males. It is apparent, that in both types of FSH-R polymorphisms no differences were disclosed between males and females.

The genotype 680 exon 10 polymorphism Asn/Asn, Asn/Ser, Ser/Ser are not different from so far published prevalence in Caucasian population.

These data provide possibility to compare the genotype characteristic of FSH-R polymorphisms for association studies in male and female reproductive disorders and for the pharmacogenetic strategy in hormonal treatment and stimulation in male and female patients.

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#### P07.055

#### Epidemiologic study of isolated gastroschisis in mexican population: 1978-2006

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Isolated gastroschisis (IGC) cluster in the offspring of very young mothers. Incidence reported range between 0.40 and 4.49 per 10000 births. Recently has been observed an increase in the frequency without a clear explanation, although several studies have identified consistent risk factors (RF). The increasing prevalence in newborns observed in a sample of the Mexican populations prompted us to analyze the secular trend for IGC and the following RF: maternal age, primigravidity, socioeconomic status, use of vasoactive medications and change of paternity. The information was obtained from the RYVEMCE; a hospital based multicentric case-control study. We studied IGC in a sample of 1'066,542 newborns analyzing time trends prevalence from January 1978 to December 2006 and the mentioned RF in both, mothers of IGC cases and controls. P <0.05 was accepted as statistical significance. We observed a total of 151 cases with IGC, 49% were males and 51% females. The risk of having a baby with IGC was 2.4 times higher in very young mothers, <20 years (3.08/10000) than mothers ≥20 (1.17/10000), OR: 3.44, IC<sub>95</sub> 2.47-4.81. An evident time trend in the incidence of IGC starting in 1993 was observed, rising from an average of 0.50/10000 during 1978-1992 to an average of 2.44/10000 during 1993-2006 (p=1x10<sup>-6</sup>). Primigravidity was more frequent in cases than controls (66.2 and 41.1%). There were not statistical differences in the use of therapeutic drugs, socioeconomic level and change of paternity. Our results confirm an increasing trend for IGC particularly related to mothers of very young age.

#### P07.056

#### The peopling of North Asia: Y and X perspectives

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To reconstruct the origin and evolution of human populations in North Asia we investigated the genetic diversity in 50 population samples (about 2000 individuals totally) using Y and X chromosome lineages. Y-chromosomal haplotypes were constructed with unique event polymorphisms (UEP) and STR markers according to Y Chromosome consortium (YCC) classification. SNP markers in a single 60 kb linkage disequilibrium region of ZFX gene was used to trace the X chromosomal population history.

The genetic diversity of Y haplogroups was quite high (0.70 - 0.95) in most populations except few very isolated groups. The proportion of inter-population differences in the total genetic variability measured by Fst statistics is 17% for binary haplogroups and 19% for YSTR. Multidimensional scaling and principal component analysis revealed four major components in North Asian Y gene pool, reflecting the presence

of Paleoasiatic (Q), Proto-Uralic (N3, N2), Eastern Asian (O, C), and Western Eurasian (R1, I, J) lineages.

X-chromosomal haplotypes in North Asia are less diverse (gene diversity within populations 0.65 - 0.80) and less differentiated ( $F_{ST} = 4\%$ ) compared to Y lineages.

The population clustering by X and Y gives, to a first approximation, a similar picture, and matrixes of genetic distances between populations for X and Y haplotypes significantly correlates.

The age of genetic diversity generation and time of population differentiation demonstrates the Upper Paleolithic origin of major Y and X lineages and post-glacial population expansions.

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#### P07.057

##### Genetic differentiation of ethnic groups of Russia evaluated by genes of hereditary disorders

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On the basis of genetic epidemiological study the prevalence of autosomal dominant, autosomal recessive and X-linked recessive disorders in 10 regions of Russia (5 ethnic groups: Russians, Maris, Chuvashs, Udmurts, Adighes) was estimated. The size of the investigated population was more than 2.5 millions of persons. Genetic differentiation between populations of different hierarchical levels by estimated loads of hereditary diseases was established. The prevalence rate of all Mendelian disorders varied in the investigated populations from 1.34 to 5.92 per 1000 persons. Genetic diversity of hereditary diseases in the investigated populations was also under our study. 199 autosomal dominant, 165 autosomal recessive and 48 X-linked recessive diseases were revealed. Most of them were rare or very rare. There were some cases of local accumulations of hereditary disorders in the investigated populations. 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 genetic diversity of autosomal disorders.

#### P07.058

##### Age-dependent genetic polymorphism frequencies and Gene - Pass

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The report highlights the results of collaborative studies of personalized anti-aging medicine and its impact into longevity and aging. Special attention is paid to the gene nets of cardiovascular diseases, renin-angiotensin system, diabetes mellitus, osteoporosis etc. Polymorphic variants of at least some particular genes such as ACE, AGT, PAI1, MTHFR, APOE, also as metabolic genes, like GSTs and other oxidative stress markers (NOS) are considered as the most plausible candidates of the genes crucial for aging. Molecular analysis of these particular genes supplemented with relevant metabolic genes testing, responsible for efficiency of detoxification system might have substantial contribution into personalized anti - aging medicine. The data on allele frequencies distribution for 10 differenet genes in newborns (106), 119 middle age and 148 old people over 69 are presented. Relevant gene testing supplemented with its adequate sophisticated interpretation and constructive recommendations might have substantial contribution to human health and should be considered as a new highly promising tool in anti-aging medicine "Gene - pass " term is suggested for the individual DNA data bank reflecting increased personal susceptibility to these common disorders. Tremendous impact to its practical application could be achieved through wide scale application of biochip technology. The latter are already available or are in progress for a number of multifactorial diseases. Special attention is paid to Genetic Pass of Reproductive Health - a version of Genetic Pass adjusted to the needs of pregnant woman. Life in harmony with personal gene makeup remains indispensable prerequisite of longevity and good health..

#### P07.059

##### Genetic demography analysis in urban population in Buryatia Republic

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Ulan-Ude city is center of Buryatia Republic in Trans-Baikal region of Russia. It was founded about 300 years ago. From that time, both Russians and Buryats live there. We have studied genetic demography characteristics in the population of Ulan-Ude and have found some differences between these two ethnic groups. First of all, migrations pattern for Buryats is bordered mostly by Buryatia region, whereas for Russians migrants from distant regions of Russia are typical. However, fraction of homo-local marriages in Buryats is twice lower than in Russians. Frequency of homo-ethnic marriages in the two ethnic groups reflects their portions in the city population. Assortative marriage index by ethnicity was 87% for Russians, being slightly higher than for Buryats (81%). At the same time, assortative marriage indices by locality were similar for both ethnic groups. Total inbreeding estimated by isonymy index was 0.0007 for Buryats and 0.0005 for Russians. These estimates were lower than those indices for other Siberian peoples (Tuvians, Northern Khantys). Mean age of the first marriage was in Buryats 25.9 years for men and 24.72 years for women, whereas in Russians it was 24.86 and 22.69 years, respectively.

#### P07.060

##### Genome-wide association analysis identifies multiple loci associated with normal variation in height

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There are many single gene disorders that affect stature, but little is known about the genetic variants that explain normal variation of adult height. The availability of genome-wide association data offers new opportunities to identify the genes involved in normal growth. Recent meta-analyses of genome-wide association studies (GWAS), using up to 16,000 individuals, have identified 22 independent loci associated with height ( $p < 5 \times 10^{-8}$ ). The GIANT consortium has now extended these analyses, using imputation methods, to combine association results from 13 GWAS, with a total sample size of >32,000 individuals.

Initial meta-analysis identified 111 independent loci with  $p < 1 \times 10^{-5}$  and 50 with  $p < 5 \times 10^{-7}$ . Confirmed loci implicate a wide range of molecular processes involved in normal growth. These include Hedgehog signaling (*PTCH1*, *HHIP*, *IHH*), chromatin remodeling (*SCMH1*, *HMG2A*), and basic cell cycling (*CDK6*, *ANAPC13*). Some of the variants and genes have been connected to other diseases, including cancer, suggesting that variants associated with height may also influence disease susceptibility. Many of the associated loci include genes known to be involved in growth based on monogenic human studies. Other loci implicate genes previously unsuspected to have a role in growth, and represent excellent candidate genes for, as yet unexplained, growth-related single gene disorders.

Combining data from many genome-wide association studies is likely to result in the identification of hundreds of loci that influence adult height. These data should result in an unprecedented increase in our knowledge of the genetics of growth and development.

#### P07.061

##### Persons with greater individual genome-wide heterozygosity show lower increase of cortisol levels during acute psychological stress

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Aim: Genome-wide heterozygosity (GWH) was reported to affect a range of quantitative traits in humans, animals and plants. Several studies suggested that the underlying mechanism is increased compensation potential to environmental stressors in outbred individuals. In humans, psychological stress has been consistently linked to an increase in cortisol levels, which eventually impairs immune system function. We studied whether GWH status affects the increase in corti-

sol levels in persons reporting acute psychological stress.

**Materials and methods:** We studied 1,026 examinees from the Vis island, Croatia. Standardised multilocus heterozygosity (sMLH) was computed for each person from a genome-wide scan using 317,000 single nucleotide polymorphisms. Morning cortisol level was measured from the blood in all participants. Reported acute psychological stress was measured using General Health Questionnaire 30 (GHQ-30).

**Results:** GHQ-30 scores had a significant effect on the increase of cortisol ( $p<0.001$ ). All examinees were therefore categorized in 3 groups according to the increasing levels of reported psychological stress within GHQ-30. In all groups, the effect of sMLH on cortisol levels was investigated using general linear model with inclusion of potential confounding variables. In the group reporting mild stress the effect was weak and non-significant ( $\text{Beta}=-0.058$ ,  $p=0.34$ ), but with increase of reported stress levels to moderate and strong, the effect became larger and statistically significant ( $\text{Beta} = -0.125$ ,  $p=0.003$ ; and  $\text{Beta}=-0.188$ ,  $p=0.04$ , respectively).

**Conclusion:** This study implies that, in humans, the increase in individual GWH may buffer the effects of psychological stress on cortisol levels, thus protecting the physiological function of immune and endocrine system.

#### P07.062

#### GLA mutations in young adult patients with a first stroke: The PORTYSTROKE study - screening genetic conditions in PORTuguese Young STROKE patients

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#### Background

Fabry disease (FD), an X-linked lysosomal storage disorder due to mutations in the alpha-galactosidase gene (GLA), is associated with the early onset of cerebrovascular disease (CVD), most commonly of the ischaemic small-vessel (ISV) type. Atypical phenotypes have been recognised and the disease is probably under-diagnosed in the stroke population.

#### Methods

Between 1/November/06 and 31/October/07, all patients aged 18-55 years presenting with a first stroke event to any of 12 major neurology hospital departments in Portugal were offered genetic screening for GLA mutations. Strokes were classified according to usual clinical and brain imaging criteria. Mutations were detected by cDNA analysis and confirmed on genomic DNA.

#### Results

Out of a total of 625 eligible patients, 493 (78.9%) consented to the genetic analysis. Of these, 74% had ischaemic strokes, of which 28.5% were cryptogenic. In 5 patients, RT-PCR was unsuccessful. Two previously described missense mutations were identified in 12 patients: p.R118C (n=6; 3 males) and p.D313Y (n=6; 4 males). Respectively 2 patients (males) with p.R118C and 3 patients (males) with p.D313Y had evidence of ISVCVD. Two patients (1 male) had a previously undescribed 5'UTR mutation (g.1136C>T). None of these patients had a diagnosis or family history of FD.

#### Conclusions

Overall, 1.23% (95%CI: 0.25-2.21) of the patients in this cohort had a recognised pathogenic GLA mutation. Despite having been described as a polymorphic allele causing a pseudo-deficiency in plasma alpha-galactosidase activity, the role of p.D313Y in ISVCVD should be further evaluated. The biologic relevance of the g.1136C>T 5'UTR mutation requires supplementary investigation.

#### P07.063

#### Identification of Ile105Val polymorphism for GST-P1 gene in five Iranian ethnic groups: Comparison with a mix-ethnic population from Tehran

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The glutathione S-transferase P1 (GST-P1) protect cells from chemical injury and has a function in detoxification of carcinogenic compounds. The polymorphism at site of codon 105 in GST-P1 enzyme has been described, this site lies in close proximity to substrate bind-

ing for electrophilic molecule. It is demonstrated that single nucleotide polymorphisms (SNP) in various genes revealed a correlation between the presence of specific allelic variants and cancer susceptibility in diverse malignancies. Furthermore, it is shown that GST-P1 polymorphism is involved in risk of development of different types of cancer. In this study, we investigated the distribution of GST-P1 polymorphism, at codon 105, among different Iranian populations. The samples were collected from healthy population from five different ethnicity groups (Fars, Mazandarani, Kurd, Turk and Turkmen). We assessed the genotype patterns of GST-P1 among Iranian ethnic groups in five regions. Then, the data was compared with the allele distribution for above gene among Tehran population (52 samples), a mix-ethnic population. The GST-P1 genotypes, Ile105Val, were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) analysis in 269 Iranian healthy individuals. Allele frequency of GST-P1 genotype, Ile105Val, for above populations were similar, and there is only significant difference among Fars and Turkmen populations.

#### P07.064

#### Contribution of environmental and genetic modifiers to severity of the typical HFE-related haemochromatosis: a multivariate analysis

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**Background/aim:** Expression of the common p.C282Y/p.C282Y HFE-related haemochromatosis genotype depends on a balance between accentuating and reducing factors. Some of these factors have been identified, but they have mainly been analyzed independently. Here, we aimed to determine contribution of different environmental and genetic modifiers to total body iron overload.

**Method:** We studied 365 p.C282Y/p.C282Y patients (195 men and 170 women), and we used the iron removed by phlebotomy as quantitative trait (log transformed). We tested the influence of age, gender, alcohol abuse, body mass index, as well as six common variants: the 16189 T>C variant in mtDNA, the -308G>A variant in the TNF-alpha promoter, the well known duplication of the haptoglobin gene (genotypes Hp1-1, Hp1-2 and Hp2-2), and 3 SNPs located in genes involved in regulation of hepcidin synthesis (BMP2 rs23756, BMP4 rs4901474 and HJV rs16827043).

**Results:** Univariate analyses first highlighted the relation between each potential modifiers and severity of the body iron overload. Factors with a p-value lower than 25% were included in a same multiple linear regression model. Then, stepwise elimination procedures were successively performed until a model presenting only significant predictors was found. Predictors of this last model were the male sex ( $p<0.0001$ ), the greater age at diagnosis ( $p<0.0001$ ), the alcohol abuse ( $p=0.0008$ ) and the Hp2-2 genotype ( $p=0.0186$ ).

**Conclusion:** Our results underline the predominant influence of non-genetic modifiers in expression of the p.C282Y/p.C282Y genotype. Future investigations should address the influence of other genetic modifiers, and further look for gene/gene and gene/environment interactions.

#### P07.065

#### 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.

#### P07.066

#### Frequency of the Hemochromatosis gene mutations in patients with Hereditary Hemochromatosis and in control subjects from Serbia

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The most common form of hereditary hemochromatosis (HH) is HH type 1. It is an autosomal recessive iron-overload disorder associated with mutations in the hemochromatosis gene (HFE). The aim of this study was to determine the frequency of the C282Y, H63D and S65C mutations of HFE gene in the group of unrelated HH patients (n=28) and in the healthy population of Serbia (n=318).

The C282Y, H63D and S65C mutation frequencies in the healthy population were 1.6%, 15.7% and 1.6%, respectively and in the population of HH patients 25%, 19.6% and 1.8%, respectively. The frequency of C282Y homozygotes was estimated on approximately 1:3900 indicating the prevalence of HH type 1 in Serbia. Our results support previously documented gradient distribution of the C282Y mutation, in northwest to southeast Europe. They also confirm that the frequency of C282Y mutation is not related to the origin of particular population but it is more related to the geographical position of the population. This finding could be considered as yet further evidence in favor of the Viking or Celtic origin of C282Y mutation.

The frequency of the C282Y homozygotes (10.7%) is considerably lower than the frequency reported for HH patients in other European populations. It is possible that some of the analyzed HH patients carry other mutations in HFE gene, mutation in some other genes already related with HH or in some other yet not identified genes.

These results have important clinical implications for the detection and management of HH type 1 in Serbia.

#### P07.067

#### Heredity hemochromatosis and multiple sclerosis. Frequency of common HFE mutations in Czech patients with multiple sclerosis

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Heredity hemochromatosis is an autosomal recessive disorder frequently found in Caucasian population and caused by iron overload (mutations in HFE gene). There have been several reports of a relationship between HFE gene polymorphisms and multiple sclerosis (MS). MS is an inflammatory demyelinating disease of the central nervous system. In the etiology of MS, both environmental and genetic factors play a role. In this study we investigated whether HFE polymorphisms is associated with Czech MS patients. We therefore genotyped 410 MS patients and 481 healthy controls for the C282Y, H63D, and S65C mutations by PCR-RFLP analysis. None of the MS patients were identified as homozygous for S65C or C282Y mutation, and seven (1.70%) for H63D mutation. Twenty eight (6.83%) MS patients were C282Y heterozygous, seventy seven (18.78%) were H63D heterozygous, and eight (1.95%) were S65C heterozygous. Among them, we have found four (0.97%) compound heterozygotes; three patients were C282Y/H63D compound heterozygous and one was found H63D/S65C compound heterozygous. Our results showed no significant differences in the distribution of C282Y and S65C HFE mutations between MS patients and controls. However, the allele frequency of the H63D mutation was significantly lower ( $p < 0.05$ ) in the MS patients than in the control group. (Supported by grant IGA MZ 1A8713 and MSM 0021620806).

#### P07.068

#### Comparison of genome-wide homozygosity-by-descent estimates in human isolated population

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The aim of this study was to investigate several methods of marker based genome-wide homozygosity estimation. The study sample consisted of 118 members of a single extended family living on the Adriatic Croatian island of Vis, where no pedigree based inbreeding was observed, and therefore no homozygosity by descent, HBD, was expected. Individuals were genotyped for 810 microsatellite markers and a 317k Illumina chip. Five approaches were compared: the proportion of heterozygous loci (multilocus heterozygosity, MLH), two methods of moments approaches that use different weighting approaches (ADC and PLINK), and finally 2 maximum likelihood approaches, one singlepoint and the other multipoint, using a hidden Markov model for marker dependencies (FEstim). The latter is the only existing multipoint method for HBD estimation and gives estimates the closest to the true HBD values, as shown previously in a simulation study. The results indicated that the two methods of moments approaches correlated highly with MLH and hence did not bring much information about HBD. On the other hand, the two maximum likelihood approaches exhibited zero value estimates when homozygosity was likely due to chance. A total of 68 individuals (57.6%) had no HBD as defined by the FEstim, while a total of 4 individuals (3.9%) had FEstim values over 0.0625 indicating inbreeding closer than first cousins. These methods can be used to differentiate homozygosity by chance vs. HBD, which may be important in investigation of the genome-wide heterozygosity effects on quantitative and health related traits.

#### P07.069

#### A medium-throughput assay for the genotyping and primary screening of useful polymorphisms using High Resolution Melting analysis

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The High Resolution Melting (HRM) analysis uses highly sensitive fluorescent dyes specific for double stranded DNA and the Post-PCR melting curve, to characterize gene fragments and identify small sequence variants such as SNPs. The purpose of this study was to determine the performance of LightCycler® 480 High Resolution Melting master and the LightCycler® 480 Gene Scanning Software, for genotyping and gene scanning purposes in the LightCycler® 480 (LC480) System. For this we setup protocols for the analysis of the *MTHFR* c.677C>T, *FII* c.20210G>A, and *F5* Leiden variants in 60 samples previously genotyped using hybridization probes. Among these samples 20 there were heterozygotes for *MTHFR* c.677C>T, 10 for *FII* c.20210G>A and 4 for the *F5* Leiden variant. The remaining samples were wild-type homozygotes. We have also developed a protocol for the mutational scanning of Trefoil factor 2 gene (*TFF2*). Post PCR HRM resulted in the expected different melting profiles that efficiently discriminated between homozygotes and heterozygotes for the different polymorphisms tested in all samples. HRM scanning of *TFF2* exon 4 in 50 control samples from our population, allowed for the identification of a previously described SNP (rs225334; MAF: 0.39) and a novel insertion, c.68\_69insCTT (MAF: 0.05) in the 3'-UTR region. Both variants could be genotyped simultaneously in the same amplicon melting analysis.

We conclude that HRM may be an efficient and cost-effective method for the simultaneous amplification, mutational scanning and genotyping of amplicons up to 200bp, in a single step and in sets of 96 or 384 samples.

**P07.070****Development of new genetic methods for predictive testing of multifactorial diseases and maximum prolongation of the human active life (analysis of 14 genes)**

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It is well-known that diagnostics on early stages and prophylaxis of different diseases is one of the most actual problems of modern medicine. Patient usually consults a doctor on the stage when disease is already in progress. At the same time the cause of pathological process often remains unclear. In this case intensive treatment can improve state of health of patient only for sometime and makes him dependent on symptomatic drugs. Diagnostic of predisposition to the multifactorial pathologies nowadays becomes an important tool that is necessary for solution of the predictive medicine problems. The basic directions of these researches are connected to genes of cancerogenesis and cardiovascular diseases. Using pharmacy biochip specially constructed for population studies, 13 polymorphisms of 7 genes: CYP1A1(C4887A, A4889G, T6235C), CYP2D6(G1934A, DelA2637), CYP2C9(C430T, A1075C), GSTM1(del), GSTT1(del), NAT2(C481T, G590A, G857A), CYP2C19(G681A) and using RFLP method 7 polymorphisms of genes: AGT(M235T), ACE(I/D), AGTR1(A1166C), PAI1(4G/5G), GPIIa(C1565T), MTHFR(C677T), NOS3(4/5) were investigated in 3 age-specific groups from North-West Region of Russia. The frequencies of same genotypes and alleles of CYP2C9, GSTM1, GSTT1, NAT2, AGT, ACE, AGTR1, PAI1, MTHFR, NOS3 genes were different between studied groups. In our investigation was demonstrated that people who have certain genotypes of studied genes for men and for women have some metabolic advantages for their longer survival. Further, it is necessary to perform studies on various groups of different age, taking into account meta-analysis data to estimate the role of age-regulating genes and multifactorial diseases in aging.

**P07.071****Genetic structure of rural population of Kazakhstan**

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The study of genetic structure of modern populations of the man is one of key problems of genetics. Genetic and demographic information for the Kazakhs population living in the Republic of Kazakhstan is presented. The marital-migrational structure of eight districts of Kazakhstan was studied on the basis of marital recodes. The average value of ethnic marriage assortativeness was found to be 1,78. The genetic structure of rural populations of Kazakhstan is formed by respective districts and region. The mean number of children per woman constituted 4,71. Crow index of total selection ( $I_{tot}$ ) and its components ( $I_m$ ,  $I_p$ ) were 0,36, 0,08 and 0,26 respectively. The size of the portion of the population of reproductive age (34,4% of the total), family size (4,71), and the predominance of the portion of the population (49,8% of the total) under reproductive allow us to classify this population as growing. The parameters of isolation of Malecot's distance and index endogamy in eight districts of Kazakhstan are counted up. Highest local inbreeding is found out in the district of Abai (0,00103). The index of endogamy is 53,6% A significant correlation between effective population size, endogamy and ethnic diversity with a level of local inbreeding was revealed ( $r = -0,896; 0,585; -0,658$ ). Recent social and economic changes have led to an increase in general and ethnic isolation of rural populations of Kazakhstan.

**P07.072****Ethnic-Pathology: distribution of disease, in Colombian indigenous groups, genetic and environmental aspects.**

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Ethnic-Pathology: distribution of disease, in Colombian minority groups, genetic and environmental aspects.

The frequency of disease varies among populations, the reasons for this variation may be social, cultural or due to environment, but the incidence, prevalence, response to treatment and natural history of disease are also influenced by genotype, and although distinction be-

tween genetic variation and the genetic variation associated with the disease is not absolute, the phenotypic expression of certain abnormal variations in the DNA depends mainly of the environment. Colombia is a multi-ethnic country with a variety of geographical areas, therefore, the behavior of the disease does not maintain a uniform epidemiological profile in all human groups, and is likely to be established according to specific profiles frameworks that define the ethnic groups. The ethnic-pathology would be defined, as the study of disease according to the affinities of a human community framed on its own aspects biological and cultural. This paper aims to describe the demographic distribution of the disease in the Colombian indigenous groups, according to their biological determinants, and to analyze how the expression of the disease varies depending on the development of the cultural and genetic determinants of a population.

**P07.073****Male infertility risk evaluation in inhabitants of radiation polluted territories of Ukraine**

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Reproductive system has been shown to be highly sensitive to radiation. Consequently continuous low dose ionizing irradiation may cause severe sexual disorders. In this connection present research aims in evaluation quality and fertility potential of human sperm from radiation-polluted regions of Ukraine, the role of radiation component in sperm damaging being assessed.

Freshly ejaculated semen was obtained from volunteers by masturbation after 3 days of sexual abstinence. Then sperm motility, morphology and concentration were analyzed by light microscopy in accordance with WHO protocol. Although light microscopy is routinely applied for the diagnosis of male infertility, however the limitations of such analysis are well recognized. Therefore flow cytometry (FCM), which allows the simultaneous measurement of several biological characteristics at the single cell level in several thousands of cells, was used as a complementary approach for rapid identification of sperm chromatin and membrane disturbances.

The following parameters, namely apoptosis development (AD), mitochondrial membrane potential ( $\Delta\Psi_m$ ) and nuclear DNA ploidy (DP), were identified on flow cytometer PAS (Partec, Germany). AD was followed by Annexin V - Apoptosis detection Kit I (BD Pharmingen, USA),  $\Delta\Psi_m$  was measured by means of Rhodamin 123 dye and DP was quantified using propidium iodide staining.

The data received have shown the existence of specific correlations between the radiation dose accumulated by donors and the quantitative distribution of spermatozoa in subpopulations of apoptotic, necrotized, immobile and viable cells. Furthermore, the increase of sperm DNA aneuploidy and DNA fragmentation proved to be concomitant to infertility growth

**P07.074****Iranian Population Data on sixteen Short Tandem Repeat loci: STR Multiplex Assays**

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A population study on sixteen new short tandem repeat (STR) loci D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, FGA and AMEL(X/Y) was performed on 1511 unrelated Iranian.

These individuals were randomly sampled, interviewed and with their informed consent added to the sample each donating 5 ml of whole blood. Whole blood was collected into EDTA tubes which were transferred for storage at 4-8 °C.

DNA was extracted from whole-blood specimens using the standard salting out method and precipitated with ethanol. The multiplex PCR was performed using approximately 2 ng of genomic DNA in a total reaction volume of 25 µl by the multiplex kit AmpFISTR Identifier.

DNA quantitated by spectrophotometry. The DNA was amplified by PCR and separation and detection by the ABI 3130 capillary system instrument (Applied Biosystems).

All loci meet Hardy ± Weinberg expectations. The results demonstrate

that these loci can be useful for human identification in forensic cases in Iran. We must pay attention that typing success in loci ranges from 100 ± 150 base pairs is better especially with degraded DNA samples.

#### P07.075

##### Identification of a geographic area characterized by "reproductive longevity" in the Sardinia Island

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Sardinian population differs from the Italian mainland and other European populations in demographic and biological traits, resulting from the socio-economic structure and the geographical and historical isolation. Its reproductive behaviour is characterized by the historical and still present tendency to late maternity. We hypothesize that areas of "reproductive longevity" exist throughout the island, where a higher incidence of elderly mothers combines with a lower risk of perinatal death. Data regard all 1980-96 births (n=299,793), occurred in 363 Sardinian municipalities. Through a smoothed isopleth mapping procedure, we explored the spatial distributions of a late maternity indicator (proportion of 35+year-old mothers), and a perinatal mortality indicator (proportion of deaths within the 1<sup>st</sup> week of life) associated with late maternity. We drew critical isopleths to highlight "excess" areas, where the late maternity indicator exceeds the average Sardinian value and approaches its upper limit. With respect to the "non excess" area (23% of 35+year-old mothers), in the "highest excess" area (27% of 35+year-old mothers) the Odds Ratio of perinatal death was lower (1.38 vs 1.78), and the proportion of consanguineous marriages and the inbreeding coefficient were respectively from 3 to 2.4 fold higher. In conclusion we suggest that such area, located in the central part of the island and qualified for "reproductive longevity", can be target of further investigations on eventual protective mechanisms against adverse perinatal outcomes in late maternity, and of studies on the possible association between reproductive longevity and achievement of an extended life span.

#### P07.076

##### High frequency of LCHAD deficiency carriers in the northern Poland

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Long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD) deficiency is an autosomal recessive disorder of mitochondrial fatty acid oxidation. It is the most common defect of the mitochondrial trifunctional protein (MTP) complex which catalyzes the last three steps of the β-oxidation spiral of long-chain fatty acids. The gene *HADHA* for the α-subunit of MTP carrying LCHAD activity is located on chromosome 2p23. 60-86% of reported patients with isolated LCHAD deficiency have a prevalent c.1528G>C mutation.

Our earlier screening of urinary GC-MS organic acid profile and MS-MS blood acylcarnitines profile in Polish children revealed the presence of 40 patients (37 families) with LCHAD deficiency. The common c.1528G>C substitution was observed on 89% of mutated alleles. A tendency for clustering the LCHAD deficient patients in northern part of Poland, especially in Pomeranian voivodeship, was found.

The aim of our study was to identify carrier frequency of the common mutation in various districts of northern Poland to verify the probability of correlation between high number of Pomeranian patients and expected high carrier frequency of the c.1528G>C mutation carriers.

Up to now, 1096 blood samples collected on anonymous Guthrie cards, have been screened. Seven heterozygotes for c.1528G>C mutation (including 4 carriers from the Pomeranian region) were detected. No samples homozygous for the c.1528C allele were identified. The preliminary study suggests that LCHAD deficiency carriers are more prevalent in the areas around the Baltic sea than in other parts of the world.

The study was partly supported by the Polish Ministry of Science Project 0678/B/P01/2007/33

#### P07.077

##### Association analysis of G-2548A and A19G polymorphisms in the human leptin gene with obesity

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Leptin is a protein hormone which plays an important role in the regulation of body adiposity, lipid metabolism and reproductive function. It is primarily secreted by the adipocytes and its concentration in the blood is proportional to the amount of body fat. Many leptin gene polymorphisms have been found, but their association with obesity is still controversial. We tested the polymorphisms G-2548A in promoter region and A19G in intron 1 of leptin gene for association with leptin concentration in the blood and obesity. A population-based association study was conducted in the population isolate of the Eastern Adriatic island of Vis, Croatia. Three hundred and twenty randomly selected subjects from the Vis population were genotyped. Obesity was defined as  $BMI \geq 30 \text{ kg/m}^2$ . The results revealed significant association of G-2548A variant and leptin concentration in a codominant pattern ( $p=0.026$ ). The ancestral G allele which displays as a minor allele in the Vis population (frequency of 0.44) was associated with higher leptin levels. The leptin concentration was significantly higher in the obese (60.1 ng/ml, N=244), than in the non-obese group (20.7 ng/ml, N=76) and it was more than three times higher in women (44.0 ng/ml) compared to men. However, no association of G-2548A and A19G polymorphisms with obesity was found (with leptin concentration, sex and age as covariates). The results indicate that the studied polymorphisms are not relevant markers for common obesity in this isolated population, but were found to influence the leptin concentration which is an obesity-related phenotype.

#### P07.078

##### High level of genetic differentiation in Siberian populations demonstrated by ZFX haplotypes

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X-Chromosome is considered as a convenient instrument used to study genetic history of populations. Firstly, since men have only one copy of X-chromosome it is easy to determine haplogroups. Linkage disequilibrium is also greater on the X-chromosome, because only two-thirds of this chromosome manages to recombine in each generation. The size of regions with a single genetic history is expected to be larger than in autosomes, once more making it ideal for human population genetic studies. In our study we have analyzed 5 SNP (rs2238925, rs2238926, rs2238928, rs2704843 and rs2704849) in ZFX gene located in Xp21.3 locus on the X-chromosome. Human populations belonged to 11 ethnic groups residing in Siberia and Middle Asia (Russians, Altaians, Buryats, Kets, Khants, Kirghizes, Komis, Tajiks, Tuvinians, Uzbeks and Yakuts) have been studied. Altogether 1073 male individuals were analyzed. Genetic differentiation of the ethnic groups under study was estimated using AMOVA analysis. General level of genetic differentiation for the investigated populations at the level of ethnic groups amounted to 3.6%. The North Siberian population (Kets, Yakuts and Khants) is most highly differentiated ( $F_{ST} = 11.2\%$ ), for the other groups (Central Asia, South Siberia and East Europe) a degree of genetic differentiation does not exceed 1%. Structural analysis of the selected SNP revealed 19 haplotypes. Median networks demonstrate the occurrence of two haplotype clusters, which may be conditionally determined as "Mongoloid" and "Caucasoid". Linkage analysis revealed a high LD level for 9 groups, excluding Ket and Uzbek populations.

**P07.079****Population study at fifteen Short Tandem Repeat loci in the Sarajevo (B&H Capitol) residents**L. Kovacevic<sup>1</sup>, N. Bakal<sup>1</sup>, N. Pojskic<sup>1</sup>, D. Marjanovic<sup>1,2</sup>;<sup>1</sup>Institute for Genetic Engineering and Biotechnology, Sarajevo, Bosnia and Herzegovina, <sup>2</sup>Institute for Anthropology, Zagreb, Croatia.

In our previous population studies of B&H human population, we used 17 STR loci included in the *PowerPlex 16® System* and *AmpFISTR®/Identifiler®*, twelve Y-chromosomal short tandem repeats loci incorporated in the *PowerPlex® Y System*, as well as 28 Y-chromosome NRY bi-allelic markers to generate Bosnian referent database. Wishing to test our database in order to obtain specific results in various DNA analysis for the local population of Bosnian Capitol - Sarajevo, we have decided to test unrelated healthy 150 individuals (situated in Sarajevo) at fifteen autosomal short tandem repeats loci. Qiagen Dnaeasy™ Tissue Kit was used for DNA extraction from buccal swabs and bloodstains and *PowerPlex 16® System* for amplification and detection. Amplification was carried out as described previously. 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). The raw data were compiled and analyzed using Genemapper® v3.2. Deviation from Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and power of exclusion were calculated. In addition, we compared obtained Sarajevo data with the data obtained from the global Bosnian and Herzegovinian population, isolated human population from the Bosnian mountain area as well with geographically closer (neighboring) European populations. The results of this study will be used as guidelines in additional improving of investigation of recent local B&H populations, both isolated and open, initiated in our previous researches.

**P07.080****Study on a possible effect of four longevity candidate genes (ACE, PON1, PPAR-gamma, APOE) on human fertility**R. M. Corbo<sup>1,2</sup>, L. Ulizzi<sup>1</sup>, L. Piombo<sup>1</sup>, R. Scacchi<sup>2</sup>;<sup>1</sup>La Sapienza University, Rome, Italy, <sup>2</sup>CNR Institute of Molecular Biology and Pathology, Rome, Italy.

A possible effect on fertility of four genes [angiotensin 1-converting enzyme (ACE), paraoxonase (PON1), peroxisome proliferator-activated receptor gamma (PPAR-γ), and apolipoprotein E(APOE)] previously found associated with longevity was sought in order to determine whether they have a pleiotropic action at different life ages. The study population was 151 Italian subjects whose reproductive life took place at the beginning of the demographic transition (declining fertility and longer life expectancy) and who had produced a mean number of children ( $3.6 \pm 2.3$ ) such as to be still useful to detect a differential reproductive efficiency associated with different genotypes.

Of these four longevity candidate genes, only PPAR-γ and APOE appeared to have an effect on fertility, indicating their possible influence on reproductive efficiency. The PPAR-γ Pro/Ala genotype, which in a previous study (Barbieri et al. 2004) showed a positive association with longevity only in men, was found associated with a higher number of children ( $6.1 \pm 3.3$ ) than Pro/Pro genotype ( $3.3 \pm 1.9$ ,  $p=0.001$ ) only in men. Compared with the other APOE alleles, the APOE\*2 allele, considered as an allele favouring a longer life-span, was confirmed to be associated with the lowest fertility ( $p=0.03$ ). The logistic regression analysis indicated that APOE and PPAR-γ polymorphisms act as independent determinants of reproductive efficiency. These data suggest that the APOE\*2 allele may follow the model of antagonist pleiotropy, whereas the PPAR-γ Pro/Ala genotype seems to exert beneficial effects both early in life and in advanced age in a gender-specific way.

**P07.081****Polymorphism of some genes in connection with age gradation**

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The aim was to evaluate age dynamics of alleles and genotypes of APOE (C112R, R158C), ACE (I/D), PON1 (Q192R), PON2 (C311S), CAT (-262C/T), GPX1 (L198P) and MSRA (-402T/C) gene polymorphisms in group of 1627 Tatars in age of 1-109 years old.

Differentiation of total group on certain age groups was carried out by

means of CHAID algorithm from SPSS Answer Tree (v.13.0). Genotyping was performed using PCR and PCR-RFLP. Fisher's two-tailed exact rest (Statistica v. 6.0) was used for age groups comparison.

In group 36-61 years increase of CAT \*C allele frequency was observed ( $P=0.004$ ). Persons in the age of 55-77 years have significantly higher GPX1\*L allele frequency ( $P=0.016$ ). APOE\*3, ACE\*D, ACE\*D/D, PON2\*C, PON2\*C/C, CAT\*T, CAT\*C/T, GPX1\*P and GPX1\*P/\*P alleles and genotypes frequencies were considerably higher in senile group ( $P<0.05$ ). ACE\*I/\*D genotype and PON1\*R allele carriers were more frequent among long-livers ( $P=0.026$  and  $0.004$  accordingly). Thus, we have demonstrated diversity of APOE, ACE, PON1, PON2, CAT and GPX1 genes polymorphisms genotypes and alleles frequencies between different age groups. Possibly, the same polymorphic variant plays a protective role for an organism at its different age stages.

**P07.082****Generation of lymphoblastoid cell lines from frozen whole blood**

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The manufacture of a lymphoblastoid cell line from a single donor blood sample is a means of securing a permanent, expandable and renewable source of genetic and other cellular material. Once a single donor cell bank is available, study material can be widely distributed and will be available many years after the initial study for follow-on studies that may not have been originally anticipated, or which may not be possible with existing technologies. In many respects a cell bank can be regarded as a means of immortalising a very valuable study cohort and is the soundest means of underpinning a biobank and maximising its value in the long term. However, the majority of biobanks participating in organisations such as the Public Population Project in Genomics (P3G) Consortium do not currently store samples for future cell line generation.

ECACC Human Genetic Services has approximately twenty years experience in providing strategic support to genetic research throughout the UK and Europe, through the provision of a blood processing and EBV transformation and cell banking service.

In this presentation we describe the development of a new process for the generation of EBV transformed lymphoblastoid cell lines from cryopreserved aliquots of whole blood which represents a cost effective alternative to current methods involving separated peripheral blood lymphocytes.

**P07.083****No significant contribution between M470V and 5T polymorphisms and cystic fibrosis phenotype in Iranian patients**F. Mirzajani<sup>1</sup>, F. Mirfakhray<sup>2</sup>, F. Asadi<sup>3</sup>, M. Rafiee<sup>4</sup>, H. R. Kianifar<sup>5</sup>;<sup>1</sup>National Institute for Genetic Engineering & Biotechnology, Tehran, Islamic Republic of Iran, <sup>2</sup>Shahid Beheshti Medical University, Tehran, Islamic Republic of Iran, <sup>3</sup>Islamic Azad University of Tehran, Science & Research Campus, Tehran, Islamic Republic of Iran, <sup>4</sup>Tabriz Children Hospital, Tabriz, Islamic Republic of Iran, <sup>5</sup>Ghaem Children Hospital, Mashad, Islamic Republic of Iran.

The most common CFTR polymorphism, M470V, has been shown to be relatively frequent among Iranian Cystic Fibrosis patients. Whether M470V polymorphism and 5T variant have CF causing contribution in Iranian population is not clear yet and it may increase difficulties in genetic counseling. In order to compare the frequencies of these variations, 100 CFTR alleles from normal controls and symptomatic Iranian CF patients were analyzed for the presence of 5T and M470V polymorphisms using PCR-RFLP method. The frequencies obtained for M470V and 5T variants were almost the same in the studied groups, suggesting that these two polymorphisms do not have strong indication of being a disease causing polymorphism. The variation in distribution of such common polymorphisms among very diverse Iranian population deserves more investigation with higher number of samples.

**P07.084****Male infertility induced by mtDNA/Y unfavorable combination? An association study on human mitochondrial DNA**S. C. Gomes<sup>1</sup>, S. Fernandes<sup>2</sup>, R. Gonçalves<sup>1</sup>, A. T. Fernandes<sup>1</sup>, A. Barros<sup>3</sup>, H. Geada<sup>4</sup>, A. Brehm<sup>1</sup>;<sup>1</sup>Human Genetics Laboratory, University of Madeira, Funchal, Portugal, <sup>2</sup>Genetics Department, Faculty of Medicine, University of Porto, Porto, Portugal, <sup>3</sup>Centre of Reproductive Genetics A Barros, Porto, Portugal, <sup>4</sup>Faculty of Medicine,

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There is growing evidence that certain mtDNA haplogroups determine a genetic susceptibility to various disorders bringing out the interest in the possible role of mtDNA background on the phenotype expression of mitochondrial genetic disorders. An association between haplogroup T and asthenospermia has been reported and several sub-lineages of haplogroup U were associated with differences in sperm motility and vitality. The deletion of some DAZ copies gene in 10-15% of azoospermic and oligospermic patients has been reported but also present in fertile men belonging to certain Y-haplogroups. The findings of one study have rarely been replicated by studies in other populations and conflicting associations have been reported. Our focus in this case-control study is to investigate the existence of other influences, besides a weak mtDNA background, promoting male infertility. The occurrence of a specific mtDNA variant associated to a certain Y-chromosome haplogroup could represent a vital link that will compromise the sperm function and be responsible for male infertility. A group of 99 infertile men and other one composed by 90 subjects with proven fertility were selected and analysed. The frequency of the combination mtDNA-haplogroup H (especially with the CRS sequence) and Y-haplogroup R was higher in fertile than in infertile men seemingly to be favorable to fertility. On the other hand, a considerable number of infertile men belonging to mtDNA-haplogroup H (CRS) and to Y-haplogroup I, associated to a specific DAZ gene deletion pattern- 2+4d, suggests a non favorable combination to male fertility.

#### P07.085

##### Genetic risks factors for melanoma development in Latin America

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**Introduction:** Genetics of Melanoma network (GenoMEL) is focused in melanoma genetic susceptibility in Europe, Australia, North America and Israel. Nowadays GenoMEL is obtaining samples and data from Latin America.

**Objective:** To Study well-known genes for melanoma (MM) susceptibility (*CDKN2A*/ *p14arf*, *CDK4*, *MC1R*) in familial and multiple primary MM in Latin America.

**Methods:** In a total period of three years (2001-2009) patients with genetic susceptibility for MM from Latin America.

**Results:** Until now we have analysed 16 pedigrees (25 individuals) from Mexican and Uruguayan pedigrees and identified the I49T, M52T *CDKN2A* mutations in Mexico and E88X, -34G>T, G101W in Uruguay. The percentage of *CDKN2A* mutation was 100% in families with 4 MM cases (1/1 family), 25% in 3 MM (1/4), 55.5% in 2 MM (5/9), and no mutation in 2 MPM without familial history.

**Conclusion:** *CDKN2A* mutations are responsible of MM susceptibility in familial MM in Latin America. *CDKN2A* mutations with founder effect (G101W, -34G>T) were detected in Uruguay. Nonsense germline E88X *CDKN2A* mutation was detected in two unrelated Uruguayan families. M52T mutation detected in Mexico was not previously described. One MPM Mexican patient was believed homozygote for I49T by sequencing but later MLPA showed deleted exon 1 alfa.

#### P07.086

##### Variants in the Vitamin D Receptor Gene and Melanoma Etiology

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The aetiology of malignant melanoma (MM) remains unclear but it is known that both genetic and environmental factors influence the development of sporadic disease. The main reason for the increasing incidence of MM in the general population is greater sun exposure. Epidemiologic studies confirm that UV radiation is the main factor involved in the pathogenesis of the disease. Among phenotypic factors, fair pigmentation and low tanning ability are the most important risk factors. In recent years there has been a increasing interest in the

role of vitamin D and its active metabolites in MM susceptibility. Sunlight induces production of vitamin D that has been associated with antiproliferative and pro-differentiative effects in both melanocytes and cutaneous melanoma cells mediated through the vitamin D receptor. In this study we explore the interaction between sunlight exposure and vitamin D receptor gene polymorphisms in the etiology of MM.

This case-control study included 131 consecutive Spanish MM patients from the Dermatology Unit of the Gregorio Marañón Hospital and 245 control subjects frequency matched for sex and age. Phenotypic information was collected using a standardized questionnaire.

Four SNPs in the vitamin D receptor gene (VDR) were genotyped. SNPs on exons or in the putative promoter region were selected. We were able to identify several individual SNPs and haplotypes associated with tumoral characteristics such as breslow index and melanoma location. We also discuss the role of VDR variants as possible markers for MM and its aggressiveness.

#### P07.087

##### An Empirical Comparison of Meta-Analyses of Published Gene-Disease Associations Versus Consortium Analyses

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**Background:** Consortia of investigators currently compile sufficiently large sample sizes to investigate the effects of low-risk susceptibility genetic variants. It is not clear how the results obtained by consortia compare with those derived from meta-analyses of published studies. We performed meta-analyses of published data for 16 genetic polymorphisms investigated by the Breast Cancer Association Consortium and compared sample sizes, heterogeneity, and effect sizes.

**Methods and findings:** PubMed, Web of Science, and HuGENet databases were searched for breast cancer case-control association studies. Summary odds ratios (ORs) and 95% confidence intervals were calculated for three genetic models (homozygotes, heterozygotes, and per-allele comparisons), using random effects analyses. The strength of the epidemiological evidence was evaluated on the basis of recently proposed criteria. We found that meta-analyses of published data and consortium analyses were substantially based on different data. Published data by non-consortium teams amounted on average to 26.9% of all available data (range 3.0%-50.0%). The correlation coefficient of the I<sub>2</sub> heterogeneity estimates of the two approaches was 0.47 (p=0.001), and that of the OR estimates was 0.44 (p=0.002). The meta-analyses of published data and the consortium analyses both showed statistically significant decreased breast cancer risks for CASP8 D302H, and all meta-analyses were graded as having "strong" evidence.

**Conclusion:** Meta-analysis of published gene-disease associations may still have an important role in the synthesis of knowledge on the genetic basis of human diseases. The expense and complexity of consortium-based studies should be considered vis-à-vis the potential methodological limitations of synthesis of published studies.

#### P07.088

##### Recessive congenital methemoglobinemia type I in Yakutia: the frequency of disease gene and age of the mutation

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Recessive congenital methemoglobinemia (RCM) type I is defined by cyanosis due to methemoglobinemia and an isolated deficiency of NADH-cytochrome b5 reductase (b5R) in erythrocyte. This disease is rare in the world but frequent in Sakha Republic (Yakutia) of Russia. In research based on clinical evidence frequency of RCM in Yakutia amounted to 1:5677.

In previously investigation has been showed that all RCM cases here were caused by single mutation c.806 C>T in *DIA1* gene that suggested presence founder effect for this disease in Yakutia. Allelic frequency of the mutation amounted to 0.025 and calculated frequency of disease amounted to 1:1600.

The date of diffusion of the mutation has been calculated by linkage disequilibrium between disease locus and markers closed to it. The age of mutation is approximately 310 years.

**P07.089****Population genetics study of three Microsatellite Markers (D3S1358, TH01 and FGA) in two Romanian groups and their genetic relationships to European populations****A. Rodewald<sup>1</sup>, A. Kroll<sup>1</sup>, G. Cardos<sup>2,3</sup>, C. Tesio<sup>3</sup>, C. Tesio<sup>3</sup>, D. Banica<sup>4</sup>;**<sup>1</sup>*Department of Human Biology of University, Hamburg, Germany, <sup>2</sup>"Victor Babes" National Institute, Bucharest, Romania, <sup>3</sup>Faculty of Biology, University of Bucharest, Bucharest, Romania, <sup>4</sup>"Marius Nasta" Institute of Pulmonary Diseases, Bucharest, Romania.*

In this study three different DNA-Microsatellites (D3S1358, TH01 and FGA) have been analysed in a sample of 200 individuals from the Romanian capital Bucharest; this data was compared with the data from a sample of 110 individuals from Prahova Valley (Carpathian Mountains) and with other European, Asian and African populations. Further, we investigated the possible extent of genetic influences by migrations of European neighbour populations on the Romanian genetic pool.

The results reveal that there were not any significant differences in the allele frequencies from the three microsatellite markers between the panmictic population of Bucharest and the slight isolated population from Prahova Valley.

Genetic distance analysis showed a close genetic relationship with Greek population as well as Slavic populations from Poland and Slovenia.

Historically this could be the result of intense trading activities of old Thracian and Daco-Gethic tribal groups from Romanian territory and Greek population groups, who established trading colonies at the west coast of the Black Sea (actually East-Romania) during the 6th-8th century. The Slavic influence is thought to be the result of the migrations of Slavic groups in the 6th-9th century across the Danube and Carpathian regions.

The study showed a more significant genetic distance from Romanian populations to the Croatian, Austrian, Hungarian and German populations.

This data can also be used for paternity and forensic analyses in the Romanian population.

**P07.090****Evolutionary process of disease-associated oligonucleotide repeats until their expansion in humans****S. Martins<sup>1</sup>, V. C. N. Wong<sup>2</sup>, P. Coutinho<sup>3</sup>, J. Sequeiros<sup>4,5</sup>, A. Amorim<sup>1,6</sup>;**<sup>1</sup>*IPATIMUP, Porto, Portugal, <sup>2</sup>Dept Paediatrics and Adolescent Medicine, The Univ of Hong-Kong, Hong-Kong, China, <sup>3</sup>Hosp S Sebastião, Santa Maria da Feira, Portugal, <sup>4</sup>IBMC- Instituto de Biologia Molecular e Celular, Porto, Portugal, <sup>5</sup>ICBAS, Porto, Portugal, <sup>6</sup>Fac Ciências, Univ Porto, Porto, Portugal.*

More than 40 neurological and neuromuscular disorders have been identified as caused by the expansion of repetitive sequences. The study of these loci over an evolutionary timescale becomes especially relevant to understand better the mechanisms behind these human-specific pathogenic expansions. In a previous study of the ATXN3 locus, responsible for Machado-Joseph disease, we found evidence for an alternative, multi-step mechanism promoting allelic variation within the normal range, either by slippage replication of multiple CAGs or gene conversion. To gain insight into the wild-type alleles that might have been involved in the expansion process, we have now compared the flanking STR-backgrounds of different normal-sized alleles with the ancestral expanded haplotypes from the same SNP-based lineage, in 67 MJD families (42 from the GTGGCA and 25 from the TTACAC haplotypes), analysed as before. Pairwise comparisons did not show a direct correlation between the size of wild-type haplotypes and its genetic distance to expanded alleles. For lineage TTACAC, the genetic background of expanded alleles was identical to those with 20 and 28 CAGs ( $R_{ST}=0$ ); for GTGGCA, a close relationship was found between expanded chromosomes and  $(CAG)_{23}$  and  $(CAG)_{27}$  alleles. These results led us to propose the possibility of a multi-step mutation-al mechanism, underlying also the process of *de novo* expansions in MJD. Assuming that similar mechanisms may be driving other repetitive tracts, we plan to study the evolutionary dynamics at the ATXN1, DRPLA, ATXN7, KLHL1AS, PPP2R2B, TBP, and FMR1 loci. Selection was based on different allelic distribution, consensus motif and repeat configuration.

**P07.091****On the epidemiology of the *MTHFR-C677T* gene variant in Mexico: A multiregional and multiethnic study****O. M. Mutchinick, M. A. López, B. E. Sánchez;***Instituto Nacional de Ciencias Médicas y Nutrición, México, D.F., Mexico.*

It is well known the worldwide prevalence differences of the *MTHFR* gene C677T variant. Also is well known that the homozygous for the T allele, produce a deficient methylenetetrahydrofolate reductase enzyme with less than 50% activity, being this considered a risk factor for some birth defects, cardiovascular disease, thrombosis and cancer, among other health problems. In 1999 we reported a very high T allele prevalence in adult healthy Mexican female sample.

Herein we report the allelic and genotypic prevalence of the *MTHFR-C677T* variant found in 800 healthy newborns (HNB) from eight different provinces of the North, East, West, Centre and South and 440 indigenous people from five different ethnic groups (DEG) of the country.

In HNB the T allele prevalence and TT genotype range from 0.37% to as high as 0.71% and from 17.0% to 52.0% respectively, with a clear North to Southeast increasing gradient. In the DEG, remarkable differences were also observed, with the lower T allele (0.58%) and TT genotype (33.3%) prevalence in *Tojolabales* Indians, to the highest prevalence of 0.81% and 68.6%, in the *Mazateco* ethnic group. The same was observed when HNB from distinct provinces were compared. All groups studied were in Hardy-Weinberg equilibrium. The higher prevalence of the T allele in HNB in the Southeast provinces concurs with the natural setting of the ethnic groups studied. Our findings suggest that the very high prevalence of the T allele and TT genotype observed may be the result of inbreeding and advantage selection of the variant.

**P07.092****Mutation 35delG and M34T screening in *GJB2* (connexin 26 gene) in Estonian population****R. Teeek<sup>1,2</sup>, T. Temberg<sup>2</sup>, M. Kõiv<sup>2</sup>, E. Raukas<sup>2</sup>, N. Tõnisson<sup>2</sup>, M. Kull<sup>1</sup>, K. Õunap<sup>2,3</sup>;**<sup>1</sup>*Department of Oto-Rhino-Laryngology, University of Tartu, Tartu, Estonia,*<sup>2</sup>*Department of Genetics, United Laboratories, Tartu University Hospital, Tartu, Estonia, <sup>3</sup>Department of Pediatrics, University of Tartu, Tartu, Estonia.*

Hearing loss is the most common sensory disorder worldwide. Mutations in *GJB2* (connexin 26) gene are a major cause of autosomal-recessive non-syndromic hearing loss in Caucasian population. Mutation 35delG in this gene is the most common cause for early onset hearing loss. The purpose of our study was to find out the carrier frequency of 35delG and M34T mutation among Estonian population.

We have screened 524 consecutively born neonates (the investigation is planned for up to 1000 newborns born in one month in whole Estonia). Our study uses anonymous random samples of dried blood spots on Guthrie cards from mass newborn screening program. We've found one homozygote and 29 heterozygotes for 35delG mutation. We've also detected 31 heterozygotes for M34T mutation, but no M34T homozygote so far. The carrier frequency for 35delG was 1 in 18 and 1 in 17 for M34T. The theoretical incidence of homozygotes for 35delG is 1:1296 and 1:1156 for M34T. Previous studies have shown 1 in 35 35delG carrier frequency in South-Europe, 1 in 79 Central and Northern Europe, and 1 in 22,5 in Estonia on 113 samples only (Gasparini et al., 2000). Our study shows similarly the high carrier frequency of mutation 35delG among Estonian newborns. The incidence of mutation M34T is similar to mutation 35delG and it correlates well with results of our patients with early onset hearing loss. M34T is the second frequent mutation in our patient group.

This work was supported by grant GARLA 6808 from the Estonian Science Foundation.

**P07.093****Physiological splicing variants in the *NIPBL* gene****B. Puisac, M. Ciero, M. C. Gil, M. Arnedo, M. P. Ribate, J. C. de Karam, J. Gomes, S. Menao, A. Pie, F. J. Ramos, J. Pie;***University of Zaragoza Medical School, Zaragoza, Spain.*

Mutations in a regulator of the cohesin complex, the *NIPBL* gene, located in 5p13.1, are responsible for Cornelia de Lange Syndrome 1 (CDLS1, MIM 122470). CdLS is an inherited multisystem developmental disorder characterized by distinctive dysmorphic facial features,

growth and cognitive impairment, limb malformations and occasional multiple organ defects. The NIPBL gene encompasses 47 exons and produces a 9.5 kb transcript, which appears in several tissues with different level of expression. Besides, the identification of multiple transcripts in this gene suggest the presence of alternative splicing, a feature that has not been yet studied. Here, we report the first systematic analysis of the NIPBL splicing variants in human normal tissues.

NIPBL cDNA from adult brain, fetal skeletal muscle and leukocytes was amplified in various overlapping fragments, spanning exons 1 to 47. PCR products were separated by electrophoresis on agarose gel. Additional bands of different length than expected were sequenced. Several novel splicing variants bearing one or more skipped exons were found, but only the variant with skipped exon 12 was found in all tissues analyzed. This finding was subsequently confirmed by cloning. The variant with deletion of exon 43 was present only in adult brain. Our results provide a basis for more detailed studies of functional significance of these transcripts and might expand our knowledge of CDLS1.

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#### P07.094

##### **NOTCH3 mutations in young adult patients with a first stroke: The PORTYSTROKE study - screening genetic conditions in PORTuguese Young STROKE patients**

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##### Background

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), a genetic disorder due to mutations in the NOTCH3 gene, is associated with early onset of small-vessel ischaemic cerebrovascular disease. The clinical spectrum of CADASIL varies widely and the disease is probably under-diagnosed in stroke patients.

##### Methods

Between 1/November/06 and 31/October/07, all patients aged 18-55 years presenting with a first stroke event to any of 12 major neurology hospital departments in Portugal were offered genetic screening for CADASIL. Strokes were classified according to usual clinical and brain imaging criteria. Mutational analysis was limited to PCR amplification and sequencing of NOTCH3 exons 4, 11, 18/19, as these have been previously shown to contain ~80% of the mutations identified in Portuguese patients with CADASIL.

##### Results

Out of a total of 625 eligible patients, 493 (78.9%) consented to the genetic analysis. Of these, 74% had ischaemic strokes, of which 28.5% were cryptogenic. Seven different missense mutations in the NOTCH3 gene were found in 8 patients (prevalence=1.6%; 95%CI: 0.8-3.1%): p.R163W; p.P167S; p.T577A; p.G595S; p.S978R (n=2); p.H981Y; p.R1036Q. There was no significant association with any particular type of stroke. None of these patients had a previous diagnosis or family history of CADASIL.

##### Conclusion

The minimal estimate for the prevalence of NOTCH3 mutations among Portuguese young adults presenting with first stroke is at least 1000 times higher than the estimated population prevalence of CADASIL. The spectrum of pathogenic NOTCH3 mutations may be more varied than previously recognised.

#### P07.095

##### **Neuropeptide Y gene variation and association with alcohol consumption in a Spanish Mediterranean population**

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**Background and objective:** Neuropeptide Y (NPY) is a neurotransmitter widely distributed in the central nervous system. Both intraamygdalar injection and overexpression experiments in animals have demonstrated that increases of NPY in the amygdala reduces alcohol

intake and anxiety manifestations in anxious rats. In humans, some studies have associated the Leu7Pro polymorphism in the NPY with alcohol consumption, but the evidence is scarce. In the Spanish Mediterranean population, the Leu7Pro variant is not polymorphic. Thus, our aim was to identify novel exonic variants in the NPY as well as the study previously described intronic variants, and their association with alcohol consumption in this population.

**Methods:** 911 subjects (321 men and 590 women) from the Spanish Mediterranean population were recruited. Alcohol consumption and demographic and lifestyle variables were measured. Nucleotide sequence determination and SNP analyses were carried out.

**Results:** Only one exonic SNP was detected by direct sequencing (1258G>A or rs9785023; allele frequency 0.47). From the intronic markers chosen (483A>G or rs13235938, 2517A>G or rs4722342 and 7065A>G or rs 4722343), only the last ones were polymorphic (allele frequencies 0.46 and 0.40 respectively), and none of them were associated with alcohol consumption. However, the 1258G>A SNP was associated (recessive pattern) with higher alcohol intake in drinkers. This association was particularly relevant in men with a moderate intake (40±9 g/d in GG, 41±8 g/d in GA and 59±5 g/day in AA; p<0.05).

**Conclusions:** The 1258G>A in the NPY is associated with alcohol consumption in the Mediterranean population.

#### P07.096

##### **Splitting of large and complex pedigrees for linkage analysis of quantitative traits**

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Pedigree splitting is applied when large pedigrees are used for linkage analysis. Several algorithms of pedigree splitting have been proposed. They are adapted to MCMC, but not to Lander-Green based methods. The latter methods are sensitive to the pedigree size. None of the existing methods restrict the fragment size, and thus, they do not guarantee that all of the resultant sub-pedigrees can be efficiently analyzed by the Lander-Green algorithm. Earlier we proposed a fast automatic algorithm for splitting large pedigrees for subsequent Lander-Green based analysis (Liu et al, 2008). The algorithm is specifically aimed to deal with pedigrees obtained in disease-oriented studies in genetically isolated populations, where affected individuals are remotely related to each other through multiple lines of descent.

In contrast to pedigrees with rare diseases, in the pedigrees collected for quantitative trait analysis many close relatives having measured phenotypes and genotypes are considered as individuals of interest. We present a graph theory based algorithm for automatic splitting such pedigrees on the fragments of restricted size. This algorithm iteratively selects the clique with maximum weight of its edges from the cliques of restricted size. Weight of edge is defined as kinship between two individuals corresponding to clique nodes. The algorithm is implemented in a software package PedStr (<http://mga.bionet.nsc.ru/soft/index.html>).

We compared our program PedStr and the Greffa (Falchi et al, 2004) program and demonstrated that linkage power calculated on the base of sub-pedigrees obtained by PedStr was higher than for sub-pedigrees obtained by Greffa.

#### P07.097

##### **Association of interleukin-1 polymorphisms with periodontal disease in mentally retarded**

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Association between Interleukin (IL-1) gene polymorphisms & increased susceptibility to periodontitis were suggested. The intent of this study was determining prevalence of IL-1 α & β genotype polymorphisms among normal & mentally retarded Egyptian individuals.

Thirty normal volunteers(NV) (15-30 y) & 25 mentally retarded individuals (MR) (6-16 y) were subjected to dental examination with recording Gingival index scores (GI). IL -1 α and IL-1β loci were genotyped by standard PCR restriction fragment length polymorphism assay. Ten of (NV) showed good GI scores (70% were healthy & 30% had initial periodontitis);The remaining (20) had fair GI score (15 % with initial periodontitis, 35% had moderate periodontitis&50% with severe

periodontitis); among these individuals, 30% were genotyped positive for IL-1( 26.6 % were genotyped positive for IL-1  $\alpha$  & 3.3% for IL-1 $\beta$ ) .Non of (MR) had good GI score while 13 were fair GI (23.1% had initial periodontitis,53.8%with moderate periodontitis&23.1%with severe periodontitis) & the rest (12) had bad GI score (41.7 % had moderate periodontitis & 58.3 % had severe periodontitis). From all MR 36% were genotyped positive for IL-1( 24 % were genotyped positive for IL-1  $\alpha$  and 12 % for IL-1 $\beta$ ).

Data concluded the important role of polymorphism in genes of IL-1(IL-1 $\alpha$  (+4845) & IL-1  $\beta$ (+ 3953)alleles presence with periodontitis either in normal or MR individuals & recommended their detection to determine patient's susceptibility for periodontitis.. Evaluation of role of other potential candidate genes as contributors to periodontitis are needed .

#### P07.098

#### Frequency of the R229Q NPHS2 functional variant, associated with increased risk for microalbuminuria, in Bulgarian Roma population

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The cardio-vascular diseases are one of the leading causes for morbidity and mortality in the Western societies. Their major social and health-care system impact warrants the extensive efforts for identification of the genetic factors contributing to the predisposition to cardiovascular events. Microalbuminuria, defined as urine albumin-to-creatinine ratio of 0.03 to 0.299 mg/mg, is one of the recently recognised factors associated with increased risk of developing cardiovascular disorders in the general population. Multiple genetic epidemiological studies have shown that there are genetic causes associated with predisposition to the excretion of abnormal amounts of albumin in the urine. One of these is the R229Q functional variant in the podocin gene, NPHS2. Pereira and co-authors have shown that R229Q is associated with a 2.77-fold risk for developing microalbuminuric state even after adjustment for age, ethnicity, hypertension, obesity, and diabetes (Pereira et al., 2004). Since the distribution of this NPHS2 variant in the general population differs depending on ethnicity, we wanted to evaluate its frequency among Bulgarian Roma - an isolated population, with unique genetic background. We genotyped Roma and Bulgarian population control samples from the collection of the National Genetics Laboratory and the Molecular Medicine Center. Our results indicated that the allele frequency of the R229Q variant in Roma (3.4%; number of alleles, n=204) was not significantly different from the one we saw in Bulgarians (1.3%; n=238) and from that previously shown for Caucasians (3%, Franceschini et al. 2006).

#### P07.099

#### Investigation of the polymorphism of eight genes among Russian population

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Investigation of the polymorphic human genes sheds light on genetic structure of the different populations. Our study included eight polymorphic genes (CNB, NFATC4, PGC1A, PGC1B, TFAM, VEGF, UCP2, and UCP3). Well-known standard methods for DNA analysis were used. The study population consisted of 1057 residents of Russian Federation (female individuals - 574 aged from 16 to 19 yr, male individuals - 483 aged from 17 to 20 yr). Rates of alleles investigated are the following: CNB I - 91.5 per cent, NFATC4 Gly - 43.6 per cent, PGC1A Gly - 64.9 per cent, PGC1B Pro - 4.9 per cent, TFAM Thr - 9.1 per cent, VEGF C- 24.5, UCP2 Val -36.4 per cent and UCP3 T - 23.8 per cent

#### P07.100

#### Associations between serotonin transporter gene SLC6A4 polymorphism and level of intellectual development (IQ) of the person

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INTRODUCTION: Serotonin transporter gene SLC6A4 (17q11.1-12) is one of the basic genes, which define an overall performance of serotonergic neuromediators system. Its functional condition can be reflected on some aspects of intellectual activity of the person.

METHODS: The level of intellectual development (IQ) is certain at 250 unrelated individuals in the age of 18-35 years by nonverbal Kettel test.

According to parameters IQ examinees are divided into three groups: with a normal level of intellectual development (IQ within the limits of 90-110 points), high (above 110 points) and low (below 90 points). The analysis of genetic polymorphism 5-HTTLPR is carried out by a method PCR. RESULTS: Genotypes 1)\*L/\*L, 2)\*L/\*S, 3)\*S/\*S met frequency 1) 28%, 2) 42%, 3) 30% in group of comparison, 1) 21.4%, 2) 59.5%, 3) 19.1% in group with high parameters IQ and 1) 33.3%, 2) 41.67%, 3) 25% in group with low level IQ. The analysis of associations has shown statistically significant distinctions in distribution of frequencies genotypes of gene SLC6A4 between group of comparison and group with high parameters IQ ( $\chi^2=8.313$ ;  $P=0.017$ ), owing to increase of frequency of genotype SLC6A4\* $L$ /\*S (59.5 % against 42 % in group of comparison;  $P=0.030$ ; OR=1.418; 95%CI 1.069-1.836) in group of persons with high parameters IQ.

Is known, that the presence in genotype of allele SLC6A4\* $L$  provides high level expression of serotonin transporter gene and the high intensity of metabolism of serotonin, that is accelerating the process of pulse transmission through carrying intensification of serotonin from synaptic trough in presinaps.

#### P07.101

#### Genetic variation at nine STR loci in Russian Siberian population

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STR loci represent a rich source of highly polymorphic markers for medical, forensic and population studies. There are notable differences in allele frequencies and heterozygosities between population groups. We present here characteristics of the allelic polymorphism for nine STRs loci: D7S820, D3S1358, D5S818, D13S317, HumVWA, HumTHO1, HumF13B, HumLPL and HumCD4 in Russians (Caucasians) living in South-West Siberia.

Allele typing was performed using PCR and subsequent high-resolution PAAG electrophoresis. We have analysed 320-378 DNA samples from unrelated individuals for each STR-system. Nine alleles were identified in HumVWA (13-21 repeats, 139-171 bp), D5S818 (7-15 repeats, 134-166 bp) loci; eight alleles were note in D3S1358 (13-20 repeats, 118-146 bp), D13S317 (8-15 repeats, 169-197 bp), HumTHO1 (5-11 and 9.3 repeats, 179-203 bp), HumCD4 (7-14 repeats, 130-165 bp) loci, and seven alleles - in D7S820 (7-13 repeats, 219-243 bp), HumF13B (6-12 repeats, 169-193 bp) and HumLPL (8-14 repeats, 109-133 bp) loci. The frequency data obtained can be used for comparison to other populations.

Genotype frequency distributions were consistent with Hardy-Weinberg equilibrium for every STR-systems. The levels of observed heterozygosity were high: 0.842 (HumTHO1), 0.828 (D13S317), 0.813-0.812 (HumVWA, D7S820), 0.778-0.770 (D3S1358, D5S818), 0.701 (HumCD4), 0.694 (HumF13B) and 0.666 (HumLPL).

Polymorphism information content (PIC), discrimination power (pD), power of exclusion (W) and marching probability (pM) were performed for each locus as indicators of their discrimination potential in human identification and paternity analysis. Forensic efficiency data suggest that investigated markers are very discriminating in Russian Siberian population.

**P07.102****Comparative studying of polymorphism 3'UTR genes-candidates multifactor diseases in the Siberian populations**

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Polymorphisms in 3'UTR in *TNFAR* (rs36188382), *IL4RA* (rs2074570), *IL12B* (rs3212227), *IL12RB1* (rs3746190) and *IL12A* (rs568408) have been studied in several Siberian populations (Russians, Buryats, Tuvinians, Yakuts, 383 individuals in total). Deletion in 3'UTR gene *TNFAR* has not been revealed in all investigated groups. This can suggest that the deletion has either very low polymorphism level, or it is a mutation. For other loci, Hardy-Weinberg equilibrium was observed for all genes except *IL12A* (rs568408) in Buryats (heterozygotes excess). Russians vs all other (Mongoloid) ethnic groups have shown significant differences in allele frequencies of three SNPs: in Russians, minor allele frequencies were 0.042, 0.229, 0.189 for *IL4RA*, *IL12A*, *IL12B* accordingly, whereas in Buryats they were 0.105, 0.195, 0.380; in Tuvinians 0.109, 0.292, 0.359; in Yakuts 0.177, 0.174, 0.286. The maximum difference was observed between Russians and Yakuts and minimum difference was between Tuvinians and Buryats. In contrast, locus *IL12RB1* (rs3746190) has shown maximum differences between Tuvinians and Buryats (minor allele frequency 0.495 and 0.274 accordingly). This is additional evidence once again about complex ethnogeny and different contributions of European and Asian components in their gene pools.

**P07.103****The analysis of CFTR mutation frequencies in different populations of Russia**

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3739 healthy donors from eight regions of European part of Russian Federation were analyzed for seven CFTR mutations (CFTRdele2,3(21kb), F508del, 1677delTA, 2143delT, 2184insA, 394delTT, 3821delT) accounted for 67% of CF alleles. 15 carriers of F508del mutation, 1 carrier of CFTRdele2,3(21kb) mutation and 1 1677delTA carrier were identified in the samples of 1324 Russians from Rostov, Tver, Pskov and Kirov provinces. Three F508del carriers and one CFTRdele2,3(21kb) carrier were revealed among 780 Chuvashas from Chuvashia, two F508del carriers - among 613 Udmurts from Udmurtia and one CFTRdele2,3(21kb) carrier - in 517 Bashkirs from Bashkortostan. In the sample of 505 Maris from Mary El none of the analyzed CFTR mutations was found. The population frequency of F508del mutation is 0,00525 (0,00401÷0,00676) in Russians from European region, 0,00112 (0,00006÷0,00530) in Udmurts, 0,00192 (0,00052÷0,00496) in Chuvashas, low than 0,00458 in Bashkirs and low than 0,00296 in Maris. The differences in F508del mutation frequencies between Russians and Maries, Russians and Chuvashas, Russians and Bashkirs are significant at 5% degree, between Russians and Udmurts - at 10% degree.

**P07.104****L162V polymorphism of PPAR-alpha gene in patients with abdominal obesity**

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Aim. To evaluate the frequency of V162 allele of PPAR- $\alpha$  gene and determine the distribution of L162V genotypes in patients with abdominal obesity.

Methods. 26 males (BMI 30,58±0,80 kg/m<sup>2</sup>, waist circumference (WC) 105,56±2,23 cm), 53 females (BMI 30,28±0,59 kg/m<sup>2</sup>, WC 92,11±1,14 cm) entered the study (44,48±0,99 years). Genotypes were determined by polymerase chain reaction with subsequent restriction analysis. Biochemical components were measured by enzymatic methods.

Results. Polymorphisms were determined in 79 participants. The V162 allele frequency was 0,076. Plasma lipids, CRP, glucose, BMI and WC did not differ in man and woman regardless of PPAR- $\alpha$  genotype (woman: total cholesterol (tCh) 5,66±0,17, HDL 1,54±0,06 mmol/l, LDL 3,80±0,17 mmol/l, TG 1,48±0,09 mmol/l, CRP 5,82±0,69 mg/l, glu-

cose 5,62±0,19, BMI 30,28±0,59 kg/m<sup>2</sup>, WC 92,11±1,14 cm; man: tCh 6,24±0,25 mmol/l, HDL 1,24±0,08 mmol/l, LDLP 4,13±0,25 mmol/l, TG 3,17±0,86 mmol/l, CRP 6,82±2,62 mg/l, glucose 6,41±0,51 mmol/l).

Careers of V162 allele have higher tCh, LDLP and lower HDL values, but the difference did not reach statistical significance. The V162 careers had higher glucose levels, but the difference did not reach statistical significance (LL162 - 5,93 mmol/l, LV162 - 5,41 mmol/l, p > 0,05). No difference between the genotypic groups was observed for CRP(LL162: 6,24±1,05 mg/l; LV162: 4,69±1,40 mg/l; p > 0,05).

Conclusion. The V162 allele frequency was 0,076, similar to that reported in ischemic heart disease and diabetes mellitus patients and healthy European man. No significant differences were observed between the presence of V162 allele and changes in blood lipids, glucose levels and body fatness measurements.

**P07.105****Minor CYP1B1 involvement in the molecular basis of primary congenital glaucoma in Bulgarian Gypsies**

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Primary Congenital Glaucoma (PCG) is characterised by locus and allelic heterogeneity. Mutations in the cytochrome P450 1B1 (*CYP1B1*) gene contribute between 20-30% in cases from ethnically mixed populations and between 50-80% in populations where consanguinity is common. The Roma/Gypsies are considered to be a rare example of a founder *CYP1B1* mutation, with E387K (identified in a Slovak Roma), accounting for 100% of disease alleles. In the current study of 21 Gypsy PCG patients from Bulgaria and of 715 unscreened controls from the general Gypsy population unusual genetic heterogeneity was revealed. In the sample of affected subjects from 16 families, we identified five different *CYP1B1* mutations - four known (E229K, R368H, E387K and R390C) and one novel and potentially pathogenic (F445I), which together account for ~30% of disease alleles. Three of the mutations have been previously found in PCG patients from India. E387K was rare in both the patient and the control group (carrier rate 0.56%), indicating that its high frequency in the Slovak Roma is most likely a product of local founder effect. Sequencing of *MYOC* and genotyping polymorphisms linked to *GLC3B* and *GLC3C* did not support the involvement of these loci, previously implicated in PCG pathogenesis. The genetic basis of PCG in the Gypsies thus remains not completely resolved. The characteristics of the Gypsies as a founder population could facilitate the identification of novel unidentified PCG gene yet.

**P07.106****Evaluation of a powerful screening tool for hereditary prosopagnosia**

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Prosopagnosia (PA) or face blindness is characterized by a highly selective impairment in recognition of faces. Longest and best known is the acquired form after e.g. brain injuries, strokes or atrophy of the right-occipito-temporal cortex. Recently, we could show that (1) the congenital form, in the absence of any traumatic event, is highly frequent, with a prevalence of around 2% worldwide, that (2) it almost always runs in families, and that (3) most surprisingly segregation pattern is fully compatible with autosomal dominant inheritance. We therefore coined the term hereditary PA (HPA, Kennerknecht et al.

2006 AJMG 140A1617ff).

Diagnosis of PA is generally established by in-depth testing with standardized test batteries for visual cognition. As these time consuming tests are not suitable for large scale screening, a questionnaire based screening was introduced. In a pilot study in India and Hong Kong we could show that only a few selected questions easily identify subjects highly suspicious for PA (Kennerknecht et al. 2007, JHG 52:230ff). These tools were then applied at two Chinese Universities (Beijing Normal University, Chang Chun Taxation College). 2,000 questionnaires with a five-point-rating scale for 21 test items regarding facial and object recognition as well as some distractors were distributed. Those students with the highest scores were then invited for diagnostic interview. When starting with the highest scores, every 2nd student was a prosopagnosic. Among a total of 40 students who scored above 2 S.D. more than 1/4 (n=11, 27.5%) were prosopagnosics.

#### P07.107

##### A new approach concerning the registries for rare diseases

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The registries for rare diseases follow to obtain epidemiological information useful to understand the dimension of the problem. Knowing the prevalence of rare diseases is an essential aspect for establishing the most adequate methods for detection, prevention and management to guide the health policy and the need for specialized personnel in different regions. In addition the registries for rare diseases represent data bases useful for research. It is necessary to ask for a well-informed consent which will allow using the recorded data in registers and also for future researches. The informed consent must be obtained after a previous preparation and notification of the patient and/or his family. In this way they will have all the information connected to the research in which they are involved: results, risks, limits and benefits.

The registries for rare diseases have to be adapted, the criteria and the tracking data have to be standardized in all European countries in order to have as many cases possible. For this reason it is absolutely necessary to create a new codification system approved by all countries, which will encode all known rare diseases.

#### P07.108

##### Genetic Characterization in 301 Spanish Families Affected by Autosomal Recessive Retinitis Pigmentosa

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Introduction. Retinitis pigmentosa (RP) is a genetically heterogeneous disorder characterized by progressive loss of vision. We used a genotyping microarray (Asper) to study proved or possible recessive cases of RP, in order to optimise the molecular diagnosis of the disease.

Patients and Methods. 301 unrelated Spanish families containing 10 patients diagnosed as LCA, 96 as early onset Autosomal Recessive Retinitis Pigmentosa (onset <10 years of age) and 195 as non early-onset ARRP (onset >10 years of age). The families were also divided by genetic classification (138 autosomal recessive cases and 163 sporadic cases). All of them were analysed by a genotype microarray specific for arRP (Asper), which tested more than 500 mutations in 16 recessive RP genes: CERKL, CNGA1, CNGB1, MERKT, PDE6A, PDE6B, PNR, RDH12, RGR, PLBP1, SAG, TULP1, CRB1, RPE65, USH2A and USH3A.

Results. No mutation was found in LCA group. The mutation frequency was slightly higher in non early-onset (21,5%) than in early onset (16,6%) families but these differences weren't significatives. The allele frequencies were: CERKL 2,9% (18/602), CNGA1 0,9% (6/602), PDE6A 0,6% (4/602), PDE6B 0,3% (2/602), RLBP1 0,3% (2/602), SAG 0,5% (3/602), CRB1 0,6% (4/602) and USH2A 7,8% (47/602). There weren't significative differences between autosomal recessive and sporadic cases.

Conclusion. The using of the genotype microarray is the first step in molecular diagnosis in Spanish families with Autosomal Recessive Retinitis Pigmentosa. USH2A is the main gene responsible followed by p.Arg257ter mutation in CERKL gene in Spanish population. 95% sporadic cases are inherited as an recessive form.

#### P07.109

##### RETN polymorphisms (-420C>G and IVS2+181G>A) in the Turkish population

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Resistin hormone (RETN) plays an important role in the insulin resistance mechanism. The effects of the two variants in the resistin gene (RETN -420C>G and IVS2+181G>A) on metabolic syndrome and cardiovascular events have been studied in the Turkish population. 208 healthy individuals, 360 with metabolic syndrome and 75 subjects with heart disease were genotyped. The genotype and allele frequencies of the RETN polymorphisms in the Turkish population have been determined for the -420C>G (CC: 47%, GC: 44%, GG: 9%) and IVS2+181G>A (GG: 52%, GA: 39%, AA: 9%) polymorphisms. The genotype and allele distributions have been found to be similar to other European populations.

In our study group the IVS2+181AA genotype has been found to be associated with low HDL-cholesterol levels ( $p=0.035$ ). The AA genotype and dyslipidemia showed an association in both obese ( $p=0.012$ ) and non-obese men ( $p=0.016$ ). In the obese group, the carriers of IVS2+181AA and AG genotypes had higher triglyceride levels ( $p=0.044$ ) than the GG genotype. In men, higher triglyceride levels have been associated to -420GG genotype ( $p=0.029$ ). Furthermore, in obese men, the -420GG genotype has also been associated with dyslipidemia ( $p=0.036$ ). On the other hand, the IVS2+181A ( $p=0.0001$ ) and -420G ( $p=0.009$ ) allele carriers had lower log folate levels in the whole study population.

This study indicates that the variations in the RETN gene (-420C>G and IVS2+181G>A) may effect lipid and the folate levels. Particularly, dyslipidemia was present more frequently in IVS2+181AA and -420GG genotype-carrying obese men, hence they may further contribute to MI risk.

#### P07.110

##### The Bayash Roma: phylogenetic dissection of Eurasian paternal genetic elements

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The Bayash consist of numerous and small Romani groups speaking different dialects of the Romanian language and living dispersedly in Croatia, Hungary, Bosnia and Herzegovina, Serbia, Romania, Bulgaria, and to the lesser extent in Macedonia, Greece, Ukraine, Slovakia and Slovenia. Larger Bayash groups migrated to Croatia most likely during the 19th century, after abolition of slavery in Romania. Molecular architecture and the origin of the Croatian Bayash paternal gene pool was addressed by analysing 151 Bayash Y chromosomes from two Croatian regions, 332 Y chromosomes from Romani populations across Europe, 814 Y-chromosomes from non-Romani host populations living in Southeastern, Southern and Eastern Europe as well as with 1680 Y-chromosomes from South Asian populations. The Bayash in Croatia represent one population of largely shared paternal genetic history characterized by substantial percentage (44%) of common H1-M82 and E3b1-M78 lineages. Relatively ancient expansion signals and limited diversity of Indian specific H1-M82 lineages imply descent from closely related paternal ancestors who could have been settled in the Indian subcontinent between 7<sup>th</sup> and 9<sup>th</sup> centuries AD. Minimal time divergence of the Bayash subpopulations is consistent with their putative migratory split within Romania towards Wallachia and Transilvania. Substantial percentage of E3b1 lineages and high associated microsatellite variance in the Bayash men is a reflection of significant admixture with majority populations from the Vardar-Morava-Danube catchment basin - possibly a common paternal signature of Romani populations in Southeastern Europe. Additional traces of admixture are evident in the modest presence of typical European haplogroups.

**P07.111****Analysis of Polymorphism of the CAG Repeats in the Spinocerebellar Ataxia type 1 (SCA1) Gene in Human Populations of the Volga-Ural Region****E. Mingazova, E. Khusnutdinova, I. Khidiyatova;***Institute of Biochemistry and Genetics, Ufa, Russian Federation.*

Spinocerebellar Ataxia type 1(SCA1) is a neurodegenerative disease, is characteristic at the molecular level by CAG repeat expansions on 6p23 in the SCA1 gene. Distribution of normal alleles of CAG repeats in SCA1 gene was analyzed in populations of the Volga-Ural region, including Tatars, Russians, Maris, Udmurts, Komis, Chuvashes, three ethnogeographical groups of Bashkirs and two ethnogeographical groups of Mordovians. 13 alleles (8-12 in different populations), containing 24-37 trinucleotide repeats were found as a result of 956 DNA samples analysis. The allele frequency distribution showed two the most frequent alleles corresponding to 31CAG repeats with the frequency varied from 0,161 in Tatars to 0,328 in Chuvashes, and 32CAG repeats - from 0,152 in Mordovians erza to 0,425 in Chuvashes. Allele with large number of repeats (CAG)<sub>37</sub> was found in Bashkirs from the Abzelilovskii, Burzanskii regions, Mordovians erza with frequency of 0,008, 0,005, 0,022. Transitional alleles with number of repeats (CAG)<sub>37-39</sub> are unstable triplet repeats and can given subsequent mutation. Allele frequency distribution of SCA1 gene is significantly heterogeneity. The observed heterozygosity was the highest (99%) in Bashkirs from the Abzelilovskii region and the lowest (44%) in Mordovians erza; the average heterozygosity was 73,6%, and allows to consider this polymorphic DNA locus to be a highly informative genetic marker for populations. At present in the Bashkortostan Republic (Russia, South Ural, population 4069784 people) 10 families with progressive autosomal-dominant spinocerebellar ataxias were revealed, and in 2 of the examined families revealed the expansion of (CAG)n-repeats in SCA1 gene.

**P07.112****DNA copy number analysis in a case-control study of schizophrenia****F. Torri<sup>1</sup>, S. Lupoli<sup>2</sup>, S. Potkin<sup>3</sup>, E. Salvi<sup>1</sup>, J. Turner<sup>3</sup>, G. Guffanti<sup>1</sup>, A. Orro<sup>4</sup>, J. Fallon<sup>3</sup>, C. Barlassina<sup>1</sup>, D. Cusi<sup>1</sup>, F. Macciardi<sup>1</sup>;**<sup>1</sup>*University of Milan, Milan, Italy, <sup>2</sup>INSPE, Milan, Italy, <sup>3</sup>Dept. of Psychiatry & Neuroscience, UCI, Irvine, CA, United States, <sup>4</sup>CILEA, Segrate, Milan, Italy.*

Recent studies have highlighted DNA copy-number variations (CNVs) as a largely under-explored source of human genetic variation, which could be responsible for the development of complex disorders. According to this hypothesis, evaluation of DNA copy number in schizophrenia may yield insights into the discovery of genetic risk factors for this disease (as aberrations in genes involved in glutamate signalling suggested in a recent report<sup>1</sup>), as CNVs can also be transmitted as mendelian traits<sup>2</sup>.

We have assayed 317.511 SNPs in 172 DNA samples from a case-control study of schizophrenia, including 91 controls and 82 schizophrenics, representing the first wave of a much larger sample, using the Illumina HumanHap300 Genotyping BeadChip®. In an effort to examine individual chromosomes for structural mutation, we used two different classes of algorithm: cnvPartition (circular binary segmentation algorithm) implemented in BeadStudio v3.0.22 and quantiSNP3 (Objective Bayes Hidden Markov Model).

Statistical analyses performed on genotyping data-sets of our study revealed that the overall distribution of CNV assignments is significantly different between schizophrenic patients and controls, with the main difference observed for duplications, which are more frequent in schizophrenics than in controls.

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**P07.113****Analysis of Y-chromosomal STR haplotypes in two ethnic groups and one cosmopolitan population from Tunisia****K. Fadhlaoui-Zid<sup>1</sup>, I. Mendizabal<sup>2</sup>, H. Khodjet Ell Khill<sup>1</sup>, B. Martinez-Cruz<sup>2</sup>, M. Ben Amor<sup>1</sup>, A. Ben Ammar Elgaaeid<sup>1</sup>, D. Comas<sup>2</sup>;**<sup>1</sup>*Laboratoire de Génétique, Immunologie et Pathologies humaines. Faculté des Sciences de Tunis. Université Tunis El Manar, Tunis, Tunisia, <sup>2</sup>Unitat de Biologia Evolutiva. Universitat Pompeu Fabra. Doctor Aiguader 88, 08003, Barcelona, Spain.*

Human Y-chromosome short tandem repeat (Y-STR) markers show a high level of polymorphism and a significant degree of discrimination between individuals. Analysis of human Y-STR polymorphism has become a very useful tool both in evolutionary studies and in forensic casework, due in part to the loci being recombination free during meiosis and paternally inheritance. In order to define the Y-chromosome genetic structure in Tunisian population, 17 Y-STRs were typed in 159 unrelated healthy males in two ethnic groups ("Andalusians" and Berber) and one cosmopolitan population (Tunis) from Tunisia, using AmpFLSTR® Yfiler™ PCR Amplification Kit (AB Applied Biosystems).

Allele and haplotype frequencies, standard diversity indices, pairwise genetic distances (RST) and analysis of molecular variance (AMOVA) were calculated with the software Arlequin version 2.000. A total number of 111 haplotypes were identified by the 17 Y-STR loci in our study sample and 91 haplotypes were unique. All the groups analysed showed high haplotype diversities, the highest value being observed in the "Andalusians" sample: 0.9924 +/- 0.0104 for "Cosmopolitan"; 0.9765 +/- 0.0132 for Berbers from Sened; 0.9886 +/- 0.0131 for Berbers from Chenini-Douiret; 0.8367 +/- 0.0547 for Berbers from Jeradou and 0.9940 +/- 0.0095 for Andalusians. The highest diversity revealed for Chenini-Douiret using this Y-STR is not expected, as compared to mtDNA or autosomic markers analysis. The hierarchical analysis of variance carried out between the Berber and Arabic-speaking groups failed to demonstrate any significant differentiation between them. These results corroborate the absence of overall genetic differentiation between Berbers and Arabs in Tunisia.

**P07.114****Population data on the X chromosome short tandem repeat loci DXS9895, GATA172D05, DXS6810, DXS6803 and HPRT in Croatia****K. Crkvenac Gornik<sup>1</sup>, K. Štingl<sup>2</sup>, Z. Grubić<sup>2</sup>, I. Tonković Đurišević<sup>1</sup>, L. Letica<sup>1</sup>, M. Burek<sup>1</sup>, R. Lasan<sup>1</sup>, D. Mužinić<sup>1</sup>, D. Begović<sup>1</sup>;**<sup>1</sup>*Division of Metabolic and Genetic Diseases, Clinic of Pediatrics, University Hospital Centre Zagreb, Croatia, Zagreb, Croatia, <sup>2</sup>Tissue Typing Centre, University Hospital Centre Zagreb, Zagreb, Croatia.*

Due to its high polymorphism, the analysis of Short Tandem Repeat (STR) markers using the PCR method has become a widely applied technique in forensic individual identification, rapid detection of chromosome aneuploidies in prenatal and postnatal diagnosis and paternity testing. Until now a large number of autosomal and Y-chromosomal markers has been forensically evaluated and used for various purposes, but the application of X-chromosomal markers has played only a minor role in forensic practice so far. The X-STR loci (DXS9895, GATA172D05, DXS6810, DXS6803 and HPRT) were investigated in male (N=90) and female (N=93) population samples from Croatia. Samples were collected from randomly selected unrelated healthy individuals. No deviation from the Hardy-Weinberg equilibrium could be detected and allele frequencies showed similar distribution in male and female samples. With the exception of DXS6810 locus for which only 6 alleles were observed and PIC value lower than 0.75 was calculated, all other loci have demonstrated sufficient polymorphism and PIC value to be considered valuable in future forensic analyses.

**P07.115****Epidemiology of monogenic hereditary skeletal diseases in Rostov region of Russian Federation****R. A. Valkov<sup>1</sup>, T. I. Valkova<sup>2</sup>, S. S. Amelina<sup>2</sup>, R. A. Zinchenko<sup>3</sup>;**<sup>1</sup>*Railroad clinical hospital, Rostov-on-Don, Russian Federation, <sup>2</sup>Rostov regional clinical hospital, Rostov-on-Don, Russian Federation, <sup>3</sup>Research Center for Medical Genetics, Moscow, Russian Federation.*

Monogenic hereditary skeletal diseases have many various clinical forms and characterized by genetic heterogeneity. The main purpose of our research was determination of hereditary factor's role in common structure of skeletal diseases, and theirs prevalence at population of Rostov region of Russian Federation. 320925 people of eight area of Rostov region were examining. All hereditary skeletal diseases were dividing into isolated and syndromic forms. In the results 336 patients of 226 families are had hereditary skeletal diseases. It was 36% of all patients with hereditary diseases. The total prevalence rate of hereditary skeletal diseases in Rostov region was 1:950 persons. At that, the prevalence rate of isolated forms was 1:2700, and syndromic

forms - 1:1600. The most frequently autosomal dominant diseases were Ehlers-Danlos' syndrome -1:6400, Marfan's syndrome - 1:20000, idiopathic scoliosis - 1:20000, polydactylia postaxialis - 1:21400, syndactyly, type I - 1:26700, osteogenesis imperfecta - 1:32000. In autosomal recessive, it was Spondiloepiphysial dysplasia Tarda - 1:64200, Spondiloepiphysial dysplasia with mental retardation - 1:80200, Langer type mesomelic dysplasia - 1:107000. In X-linked, it was Aarskog syndrome - 1:53500, Coffin-Lowry syndrome - 1:53500, Proust syndrome - 1:80200. Therefore, our research is enabling to improve genetic consultation's activity, directed to decrease of hereditary disease's pressure in Rostov region of Russian Federation.

#### P07.116

##### **Smith-Lemli-Opitz Syndrome mutations in recurrent miscarriage**

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Recurrent miscarriage (RM) is defined as >3 (or >2) consecutive pregnancy losses before 20 weeks of gestation and affects 1-3% of all women. More than 40% of RM are of unknown etiology and assumed to be genetic in origin.

Smith-Lemli-Opitz Syndrome (SLOS, MIM270400) is an autosomal recessive disorder of cholesterol biosynthesis caused by mutations in the gene DHCR7. The prevalence of SLOS ranges between 1:15000 and 1:60000 in European populations, but there is a discrepancy between expected and observed incidence of SLOS mutations that might be in part explained by first trimester miscarriages of affected fetuses. Aim: 1. To compare the prevalence of common SLOS mutations in Greek women with unexplained RM and in a control group. 2. To correlate common SLOS mutations with RM in order to conclude if screening for common SLOS mutations in couples with >2 spontaneous miscarriages should be added in the evaluation of RM.

Study group: 124 women aged <41 years, with >2 consecutive first trimester miscarriages. Controls: 75 healthy age-matched women, with proven fertility. The three most common for the Greek population mutations of the DHCR7 gene (IVS8-1G>C, p.Trp151X and p.Th93Met) were studied using allele-specific PCR.

Two out of 124 women with unexplained RM were heterozygous for null IVS8-1G>C mutation. No carriers were found among the control group. This is an ongoing study.

#### P07.117

##### **Prevalence of functional SNPs in candidate genes for common diseases in four Siberian populations**

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Genetics of common diseases deals with common polymorphisms (in most cases SNPs). There are differences in the common diseases prevalence between human populations which may be explained in part by variation in frequencies of some predisposing SNPs depending on population descent. Furthermore, linkage disequilibrium is also known to be different in diverse populations. We studied twelve known candidate genes for common diseases of cardiovascular and immune systems, in four Siberian populations of different ethnic origin, namely Tuvinians, Yakuts, Buryats, and Russians (384 individuals in total). ACE, AGTR1, NOS3, GNB3, TNF and LTA, TNFRSF1A, ADRB2, IL4, IL4RA, IL12A, IL12B, IL12RB1 genes were studied. Few SNPs in each gene were picked for genotyping on the basis of known associations with common diseases and/or possible functional effect. We have found significant differences between populations in both frequencies and LD estimates for most of genotyped SNPs. Few SNP positions were not polymorphic in our samples. We confirmed the fact that some SNPs within ACE, NOS3, TNF, IL4RA genes are in strong linkage disequilibrium. Taking into account that significant differences were found between related populations of Asian origin, as well as between

Russians and other European populations, our results emphasize need for further investigations of prevalence of candidate SNPs for common diseases in different populations. Differences in functional SNPs frequencies suggest population specific character of genetic structure of predisposition to common diseases which should be taken into account during development of genetic tests for common diseases predisposition in different regions.

#### P07.118

##### **Application of MLPA in multiplex SNP genotyping for genetic epidemiology**

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A method for simple and cost-effective medium-scale genotyping in large epidemiological studies is often needed, for instance when investigating the entire genetic variation in a single or a few genes.

The multiplex ligation-dependent probe amplification assay (MLPA) was initially developed for detection of DNA deletions or duplications, but is also suitable for genotyping single point polymorphisms (SNPs). We designed an allele discrimination assay for simultaneous genotyping of at least 10-20 SNPs in a large number of samples using the MLPA technology. Based on the MLPA probe design protocol we constructed 3 probes per SNP: 2 allele specific and 1 locus specific, which after ligation were PCR amplified and size-separated using capillary electrophoresis. In addition to the template specific sequence each of the MLPA probes contain a variable length stuffer sequence enabling both SNPs and alleles to be discriminated by size, and a primer specific sequence common to all probes, thus permitting multiplex PCR using only one primer set.

We applied the method on two genes for which the genetic variation was covered by 12 and 19 tagging SNPs, respectively. Genotyping results were verified using CEPH controls with known genotypes.

Applying the MLPA method will enable most labs to genotype SNPs in a medium scale without investing in new lab equipment as the method only requires a PCR machine and a capillary electrophoresis system.

#### P07.119

##### **Large scale screening for spinal muscular atrophy (SMA) - effect of ethnicity on frequency of exon 7 deletion v.s duplication**

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Approximately 95% of SMA patients have homozygous deletions of exon 7 and/or 8 of the SMN1 gene and 5% are compound for deletion of exon 7 and a point mutation. The carrier frequency varies between 1:150 and 1:35.

We undertook a survey to assess the carrier rate among healthy individuals with no family history, evaluate the false negative rate (individuals with three copies of exon 7), and determine any ethnic differences.

We analysed data from two medical centres in Israel that conduct carrier screening among the normal population using the MLPA kit. We studied the copy number of exons 7 and 8 and divided the subjects into six ethnic groups: Ashkenazi, North African, Iranian/Iraqi, Yemenite, Balkan, other Jewish. Statistical analysis was performed using chi-square.

Between February-October 2007, 7308 subjects were tested in Maccabi clinics and 1729 at Rabin Medical Center. The carrier rate (deletion of exon 7) was 1:62 and was not statistically different among the various ethnic groups.

Duplication of exon 7 was found in 1 in 9 individuals - a false negative rate of 5.5%. There was a significant difference between the ethnic groups: 12% among Ashkenazim, 4.4% among North African Jews and 6%-8% in other groups ( $p<0.001$ ). This difference was also found for duplication of exon 8.

This study emphasizes the importance of determining the false negative rate for each ethnic group as it may vary markedly. The discrepancy between the rates of exon 7 deletions vs. duplications may be explained by the genetic disadvantage of deletions.

**P07.120****Prevalence of CAT-interruptions in (CAG)n-repeat region in the gene for SCA1 in Siberian populations.**

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Spinocerebellar ataxia type 1 (SCA1) is a rare autosomal-dominant neurological disorder caused by expansion of an unstable (CAG)-repeats in gene for ataxin-1 (6p22-23). Normal repeat tract (in healthy people) consists of 6-37 triplets with 1-3 CAT-interruptions whereas mutant alleles contain pure repeat tracts from 39-70 CAG. CAT-interruptions are thought to serve as stabilizing factor.

We have studied frequency of alleles without CAT-interruptions among normal size alleles from different Siberian populations (855 individuals): Yakuts (the highest level of SCA1 accumulation) and Buryats, Tuvinians, Russians, Tatars, Kirghiz, Altaians and Khantys (without SCA1 accumulation). It is the first estimation of prevalence of homogeneous (CAG)n of normal length in SCA1 gene in different ethnic groups of Siberia with distinct contribution of European and Asian components in their gene pools. *SfaN* I restriction method was used to identify the interruptions. High frequency of chromosomes with normal homogeneous CAG repeat in Yakuts (15.66%), Tuvinians (17.9%), Kirghiz (13.57%), Altaians (9.56%), Buryats (9.51%) and rather low prevalence of such individuals among Tatars (7.15%), Russians (3.75%) and Khantys (2.83%) is revealed. Thus, high frequency of individuals without CAT-interruptions in normal length alleles in the populations without SCA1 accumulation has been shown. This finding puts a question concerning role of CAT-interruptions in (CAG)n-expansion in the gene ataxin-1.

The study was supported by RFBR grant N 06-04-49086.

**P07.121****Simulation of dynamic spread of Triplet Repeat Expansion Diseases in human populations on example of spinocerebellar ataxia type 1**

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Spinocerebellar Ataxia Type I (SCA1) is an autosomal-dominant neurodegenerative disorder caused by expansion of unstable CAG codon in *Sca1* gene that leads to abnormally long polyglutamine chains in nuclear matrix protein ataxin 1. SCA1 is frequent in Yakut populations, with 1.2% and 0.3% prevalence in Abyisky uluses (northern Yakutia) and Ust-Aldansky uluses (central Yakutia), respectively, in 2003. We developed the computational model in RAD environment Borland Delphi 7 for simulation of dynamics of SCA1 in population, and verified it with the epidemiological data from the two separate Yakutia regions. The program is designed for simulation of SCA1 evolution in interactive mode. The user defines the initial parameters, such as population size and number of mutation carriers, as well as the interactive variables (demographical coefficients) - birth rate, death rate, migration and nuptiality, which can be adjusted during simulation. Other variables include: threshold number of CAG repeats, probabilities to inherit the mutation from mother/father, average expansion of trinucleotides tract during inheritance and reduction in number of mutant progenies due to genetic counseling. The model enables prediction of the length of CAG repeats, age of onset and life expectancy in individual mutation carriers in the lineage, and the prevalence of disease in population. Comparison of the data produced in simulation with the real values in existing populations was used to verify the computational model. This program provides flexible and highly visual way to predict dynamic changes in prevalence and spectrum of clinical characteristics of SCA1 in population in different demographic scenario.

**P07.122****Association analysis of Tryptophan Hydroxylase-1 gene (TPH1) and suicidal behavior in Russian population**

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Suicide is an important public health problem, ranking among the top 10 causes of death for individuals of all ages. There is strong neurobiological evidence showing that serotonergic system dysfunction predisposes to suicidal behavior. Tryptophan hydroxylase, the rate limiting enzyme in serotonin biosynthesis, is one of the most important regulating factors in the serotonergic system. The aim of our study was to examine the association of four single nucleotide polymorphisms (rs 4537731, rs211105, rs1800532 and rs7933505) of TPH1 gene and attempted suicide. DNA samples of 312 cases (101- male, 152- female), who had suicide attempts, and 346 control subjects (194- men, 152- women) from Bashkortostan (Russia) were genotyped using PCR-RFLP technique. The distribution of allelic and genotype frequencies was in accordance with the Hardy-Weinberg equilibrium. No significant differences in either allele or genotype frequencies of rs1800532 and rs7933505 polymorphisms of TPH1 gene were found between suicidal and control groups. In a male group there was a tendency of underrepresentation of the \*G/\*G genotype of rs 4537731 polymorphism in suicide group compared to that in control group (11% vs 21%; X<sup>2</sup> = 3.81; p = 0.05; df = 1). For the females \*G/\*G genotype of rs211105 polymorphism was reported to be protective marker of suicidal behavior (X<sup>2</sup> = 5.66; p = 0.017; df = 1; OR = 0.226; 95%CI 0.06-0.85). The results suggest sex-related differences in the contribution of the TPH1 gene to susceptibility for suicidal behavior.

The work was supported by RSCI grant 06-06-00163.

**P07.123****New candidate gene *B4GALNT1* is not associated with type 1 diabetes**

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Monosialo-ganglioside (GM2-1) can be found on the pancreatic β-cell membrane. GM2-1 has recently been shown to be a target for ICA and GAD autoantibodies associated with type 1 diabetes mellitus (T1DM) development. Beta-1,4-N acetyl-galactosaminyl transferase 1 (GalNAc-T) is the enzyme involved in the biosynthesis of GM2 gangliosides. GalNAc-T catalyzes the transfer of GalNAc into GM3 by beta-1,4 linkage resulting in the synthesis of GM2. Enzyme beta-1,4 GalNAc-T is coded with *B4GALNT1* gene located on chromosome 12. The purpose of this study was to examine the relation of *B4GALNT1* gene tag single nucleotide polymorphisms (tagSNP) with susceptibility to T1DM. Two analyzed *B4GALNT1* tagSNP-s, rs1008314 and rs715930, capture 100% of *B4GALNT1* common variation at the r<sup>2</sup>=0.8, based on the HapMap data. We performed case-control (213 patients and 199 control subjects) and family-based (202 families) studies in Croatian population. Case-control study did not observe an association with T1DM (rs1008314, p=0.3001; rs715930, p=0.5256). Also, transmission disequilibrium test did not detect any discrepancy from the expected minor allele transmission (rs1008314, p=0.6547; rs715930, p=0.4986) nor from the haplotype transmission from parents to affected child. This is the first ever reported association study to examine *B4GALNT1* gene polymorphisms with T1DM. We did not find any evidence to support our hypothesis of *B4GALNT1* T1DM association.

**P07.124****Quantitative Epidemiology of the Haemoglobinopathies Suggests a Molecular Model of Complex Disease Based on Variability in the Assembly of Hetero-Dimeric Molecules**

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Between 1998 and 2008 we conducted a comprehensive population based Haemoglobin testing programme. Lately we integrated the

phenotypical based testing with genotyping using low cost DNA sequencing. During this period of time, the birth rate declined from 11.7 / 1,000 to 10.3 / 1,000 and the immigrant population of Malta increased ten fold. Two  $\alpha$  globin variants Hb St. Luke and Hb Setif have been found among 0.2% of Maltese. The proportion of the variants was between 3.9% and 17.5%, depending on the effect of the mutation on the assembly and the co-current  $\alpha$  or  $\beta$  thalassaemia;  $\alpha$  thalassaemia is found among 1.3 % of neonates. Several  $\beta$  globin variants were detected including Hb Marseilles and Hb S. The Hb Valletta was always found in very tight linkage disequilibrium with the  $\gamma$  globin variant Hb F Malta I although a presumed "hot spot" of recombination has been assumed in between them.  $\beta$  Thalassaemia heterozygotes amount to 1.8% the population with 4  $\beta$  globin gene mutations accounting for over 95%. The quantitative data suggested a model based on the differential assembly of molecular subunits into hetero-dimers albeit from non-syntenic variant genes that accounted for a broad range of variant allele expression between less than 5% as in Hb S combined with an  $\alpha$  thalassaemia or around 100% as in Hb S homozygotes or the Hb S-  $\beta^0$  Thalassaemia compound heterozygosity. The globin model appears broadly applicable to a variety of possibly complex conditions associated with multiple variant genes having quantitative effects.

#### P07.125

#### Genetic analysis of a large French Canadian Tourette Syndrome family

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Tourette Syndrome (TS) is a complex neurodevelopmental disorder principally characterized by chronic motor and vocal tics and often associated with other behavioral abnormalities. Despite evidence of a strong genetic component, little is known about the genes predisposing to TS. We conducted a genomewide linkage analysis in a large French Canadian (FC) TS family comprising nine affected individuals and exhibiting an apparent autosomal dominant mode of inheritance of the disorder. Five-hundred markers with an average marker density of 8cM were genotyped. Multipoint linkage and haplotype analyses were performed using GENEHUNTER and SIMWALK2 programs. Multipoint linkage analysis of the genomewide scan revealed four chromosomal regions (2q, 6q, 8q and 13q) with LOD scores greater than 1.5 using an "affected-only" approach. Subsequent fine-mapping of these regions resulted in a single significant linkage peak (LOD>3.0) on chromosome 2q. Screening of candidate genes is needed in order to identify the causative TS gene in this family.

#### P07.126

#### The sex specific effects of UCP2 and 3 promoter polymorphisms in Turkish population

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UCP2 and UCP3 genes are highly homologous, yet they are expressed in different tissues. UCP2 is expressed in white adipose, heart, pancreas and kidney, whereas UCP3 is expressed in skeletal muscle and heart preferentially. Promoter region polymorphisms in UCP2 and UCP3 genes (-866G/A and -55C/T respectively) have been previously associated with obesity, diabetes and lipid profiles in different cohorts. To clarify the contribution of these polymorphisms to dyslipidemia and related conditions, we investigated the associations with the lipid, blood pressure and anthropomorphic measurements, with regard to diabetes and sex status. The study population consisting of a large (n=1975) representative cohort of Turkey ('Turkish Adult Risk Factor Study-TARF study, mean age=54.6 ±11.5), was genotyped using sequence specific Taqman probes. The ANOVA t-test was used to compare the differences in continuous variables among study subjects subdivided by sex and/or diabetes status. Continuous variables (having P values

≤ 0.05 in NOVA t-test) were tested further by univariate analysis using Bonferroni correction.

The genotype distributions for both UCP3-5C/T and UCP2-866G/A were in accordance with Hardy-Weinberg equilibrium. The resulting frequencies were 0.75 for UCP2 -866G allele, whereas 0.78 for UCP3-5C. After Bonferroni correction the statistical results were as follows: In male subjects, UCP3-55TT genotype was associated with decreased total cholesterol ( $p=0.028$ ) and diastolic blood ( $p=0.024$ ) pressure levels, while it was associated with higher fasting glucose (0.0001) and logHOMA-R ( $p=0.032$ ). In female subjects, UCP2-866AA genotype was associated with decreased levels of triglyceride ( $p=0.021$ ) and ApoB levels ( $p=0.027$ ).

In conclusion, the UCP3-55C/T and UCP2-866G/A promotor polymorphisms have gender specific phenotypic effects on human metabolism.

#### P07.127

#### UGT1A genetic polymorphisms in São Miguel population (Azores): implications for pharmacogenetic studies

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UGT enzymes are responsible for glucuronidation and detoxification of endogenous and exogenous compounds. Homozygosity for a polymorphism in the *UGT1A1* TATAA box promoter causes Gilbert's syndrome. This sequence contains six TA repeats (UGT1A1\*1), whereas seven repeats (UGT1A1\*28) imply reduced gene expression. In *UGT1A6*, two missense mutations result in three alleles: UGT1A6\*1 (T181-R184), UGT1A6\*2 (A181-S184) and UGT1A6\*3 (T181-S184). UGT1A1\*28 and UGT1A6\*2 are associated with reduced enzymatic activity.

Here, we determined *UGT1A1* and *UGT1A6* polymorphisms prevalence in São Miguel population (n=469 healthy individuals), and investigated *UGT1A1* association with *UGT1A6* polymorphisms. In *UGT1A1*, we identified five genotypes: 0.4% (TA)<sub>5</sub>/(TA)<sub>6</sub>, 50.5% (TA)<sub>6</sub>/(TA)<sub>6</sub>, 39.7% (TA)<sub>6</sub>/(TA)<sub>7</sub>, 9.2% (TA)<sub>7</sub>/(TA)<sub>7</sub> and 0.2% (TA)<sub>6</sub>/(TA)<sub>8</sub>. Five and eight TA repeats are found only in African-ancestry individuals. These alleles confirm our previous results on São Miguel genetic ancestry, an admixed population composed of European, Jews and Africans. UGT1A6\* genotype frequencies were 47.5% (\*1\*1), 36.2%, (\*1\*2), 7.5% (\*2\*2), 4.7% (\*1\*3) and 4.1% (2\*3). A strong association between UGT1A1\*28 and UGT1A6\*2 alleles was observed, since 81.4% homozygous for UGT1A1\*28 were also homozygous for UGT1A6\*2. Overall, 6.7% were homozygous for both UGT1 polymorphisms, and 39% had at least one variant allele for UGT1A1\*28 and UGT1A6\*2. These highly prevalent polymorphisms result in modified expression and activity of UGTs, may influence susceptibility to cancers and predispose to side effects of drugs, such as irinotecan. Currently, we are analyzing three missense mutations in *UGT1A7*, to evaluate the extension of linkage disequilibrium between *UGT1A1*, *UGT1A6* and *UGT1A7*. Funded by Azorean Government (M1.2.1.I/003/2005). PRP has PhD grant SFRH/BD/27453/2006.

#### P07.128

#### Identification of the rare UGT1A1\*36 allele in a Caucasian family of the Azores (Portugal)

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The role of UDP-glucuronosyl transferase 1A1 (UGT1A1) as a predictor of toxicity in cancer patients receiving irinotecan lead to the identification of a rare UGT1A1 genotype in a 69 years old female of Azorean ancestry.

The genotype UGT1A1\*36/UGT1A1\*28 (TA<sub>5</sub>/TA<sub>7</sub>) was identified in the index case and after informed consent, three siblings (2 females-F and 1 male-M) were investigated. DNA extraction was performed from peripheral blood cells. Amplification of region of interest was performed by PCR using specific primers; forward primer was labelled for subsequent fragment analysis. Genotyping of (TA) repeats in this region

was performed submitting PCR products to capillary electrophoresis. Analysis revealed the following genotypes: TA<sub>5</sub>/TA<sub>6</sub> (1F; 1M) and TA<sub>6</sub>/TA<sub>7</sub> (1F).

TA<sub>5</sub> allele frequencies of 0.044, 0.017 and 0.000 have been identified for African-American, Caucasian and Japanese, respectively. To our knowledge, there is only one Portuguese report with the identification of an individual with this genotype. Due to the low frequency of the TA<sub>5</sub> allele on Caucasian population, HLA typing was performed in order to evaluate putative ethnic influences. HLA alleles for loci A, Bw, Cw, DRB1 and DQB1 were typed for all the individuals (SSP, SSO and SBT). HLA extended haplotype inferred for the proband: A\*0102-B\*5801-Cw\*1802-DRB1\*1301-DQB1\*0603 revealed alleles that are extremely rare in Caucasians (e.g. Cw\*1802) suggesting that this family may have had influences from other ethnic groups. The Azores archipelago was populated mainly by the Portuguese however, settlers from other European, African and possibly Asian countries, are well known in these islands history.

#### P07.129

#### Are the Moravian Valachs of Czech Republic the Aromuns of Central Europe? Model population for isolation and admixture

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Moravian Valachs of Czech Republic are one of the most distinct ethnic groups from Central Europe. Related to similar populations in Poland and Slovakia, they emerge at the end of 15th century, as the north-westernmost prominence of migration that started 250 years earlier in northern Romania. Being predominately highland sheep herders and of putative Romanian origin, they represent a Central European analogue of Balkan Aromanian populations. We have gathered Y-chromosomal, linguistic, ethnographic and historical data for this population and compared them with surrounding as well as with east European populations.

Linguistic data show specific parts of shared vocabulary of Romanian origin between several pastoral groups in Central and Eastern Europe. Comparing genetic and linguistic pairwise distance matrices (Mantel test) in these populations did not reveal any significant correlation. Thus we confirmed that plain geographical distance still plays the major role in genetic distances between populations in Europe. From our further analysis it is clear, that the Moravian Valachs, after at least five centuries of admixture, are not overly genetically different from surrounding populations. On the other hand, from the point of view of intra-population diversity, they are much more similar to isolated Balkan populations (e.g. Aromuns) than to Central European populations.

#### P07.130

#### Haplotype profile of vitamin K epoxide reductase (VKORC1) as determinant of warfarin sensitivity in Roma population

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Oral anticoagulants, including warfarin and acenocoumarol, are the most widely prescribed drugs for treating of thromboembolic disorders. Complications with coumarin therapy include the narrow dosage window, the broad variation of interindividual and interethnic drug requirement, and the relatively high incidence of bleeding. Coumarins target blood coagulation by inhibiting the vitamin K epoxide reductase complex (VKORC). Recently, three main haplotypes of VKORC1 \*2,\*3,\*4 have been observed, that explain most of genetic variability in warfarin dose among Caucasians. The aim of the work was to study the VKORC1 haplotype profile of Roma population in Hungary, and compare with results of the average Hungarian Caucasian population. G-1639A, G9041A, C6009T single-nucleotide polymorphisms were determined for VKORC1 haplotype-tagging. A total of 455 unrelated Roma and 237 Hungarian controls were haplotyped. The genotypes were analyzed by PCR-RFLP assay and direct sequencing. Our study revealed significant difference in the prevalence of VKORC1\*2 and VKORC1\*3 haplotypes between the Roma and average Hungarian

population ( $p<0.01$ ). The H7 and H8 haplotypes, requiring high warfarin doses are predominant in Roma population were 49% together, while H1 and H2 haplotypes, requiring low warfarin dose were the predominant in Hungarian average samples. Furthermore, 21.8 % of Romans were homozygous for the H7 and H8 haplotypes and an addition 3% were accounted to H9 haplotype. Results presented here shown high variability of the Roma people, which can have therapeutic consequences.

#### P07.131

#### High prevalence of Wilson disease in a small mountain village in Romania

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Wilson disease (WD) is an autosomal recessive disorder caused by a defect in ATP7B gene, coding for a metal transporting P-type ATPase, resulting in copper overload mainly in liver and brain. Increased number of Wilson disease patients in a small mountain village next to Bran region led us to initiate a population screening. We firstly screened for mutation in five WD patients from five apparently unrelated families. Direct sequencing of all 21 exons within ATP7B gene was performed for all five WD patients. Mutation analysis revealed that each of them was homozygotes and compound heterozygotes bearing three mutations, one was frameshift mutation (P767P-fs) and two were missense mutations (H1069G and K832R). Up to now we screened 20 relatives of the five WD patients and we will screen a total of 152 autochthonous inhabitants to the third generation originating from the same village, in order to find the total number of carriers for these mutations. The high number of mutations and the homozygous/compound heterozygous state made correlation between genotype and phenotype difficult. The high prevalence WD indicates the need for health education intervention, genetic counselling and newborn screening for Wilson disease in this region.

#### P07.132

#### Allele frequencies for CYP1A1, CYP2D6, CYP2C9, CYP2C19, GSTT1, GSTM1, MTHFR, MTRR, NQO1, NAT2, HLA-DQA1 and AB0 genes in native Russians

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Xenobiotic-metabolizing genes (e.g. Cytochromes P450, GST, NAT2, and NQO1), folate metabolism genes (e.g. MTHFR, MTRR) and major histocompatibility complex genes (e.g. HLA-DQA1) play multiple role in the organism functioning. In addition, AB0 is the most clinically significant high-polymorphic gene in transfusion and transplantation medicine. Epidemiological data shows that allele frequencies of these genes exhibit ethnic and geographic diversity. Besides, little is known about frequency distribution of the major polymorphic variants in native Russians.

We developed biological microchips which allow to analyze a spectrum of allelic variants in twelve different genes such as CYP1A1, CYP2D6, CYP2C9, CYP2C19, GSTT1, GSTM1, MTHFR, MTRR, NQO1, NAT2, HLA-DQA1 and AB0. Using this composite methodological platform we have studied 352 DNA samples from healthy native Russian volunteers. For the first time we have received the allelic frequencies of above mentioned genes. The allelic frequencies of gene polymorphisms that we studied are close to allelic frequencies observed in some European populations, as published earlier. These data were used in comparative studies to determine predisposition to psoriasis, colorectal cancer, lymphoma and leukemia in adults and to childhood leukemia. The HLA-DQA1 and AB0 allele frequencies were used to estimate forensic population parameters for these loci.

**P07.133****The frequency of XRCC1 DNA repair gene A399G polymorphism in the Western Anatolia****T. Sever, S. Pehlivan;***University of Gaziantep, Faculty of Medicine, Department of Medical Biology, Gaziantep, Turkey.*

The aim of our studies, to determine of XRCC1 gene at codon 399 region frequencies of polymorphisms in healthy Western Anatolia population. We aimed XRCC1-399 polymorphism frequencies and the genotype distribution, with respect to the polymorphic codon 399 of XRCC1 gene by PCR-RFLP (Msp I Restriction Endonuclease enzyme) in 100 healthy individuals from the region of Izmir, Turkey. The following genotype frequencies were observed in the Western Anatolia population: A/A 44%, A/G 41% and G/G 15%. The frequency of A allele is 64.5% and the frequency of G allele 35.5%. The presence of the Adenine and Guanine allele frequencies in various populations such as USA, England, Caucasians, Korean and Chinese populations are similar to our results according to the published data.

**P07.134****Phylogeography of the human Y chromosome haplogroup E3a****F. Cruciani<sup>1</sup>, B. Trombetta<sup>1</sup>, D. Sellitto<sup>2</sup>, C. Nodale<sup>1</sup>, R. Scozzari<sup>1</sup>;**<sup>1</sup>Sapienza Università di Roma, Rome, Italy, <sup>2</sup>Consiglio Nazionale delle Ricerche, Rome, Italy.

The Y chromosome specific biallelic marker DYS271 defines the most common haplogroup (E3a) currently found in sub-Saharan Africa. A sister clade, E3b (E-M215), is rare in sub-Saharan Africa, but very common in northern and eastern Africa. On the whole, these two clades represent more than 70% of the Y chromosomes of the African continent. A third clade belonging to E3 (E3c or E-M329) has been recently reported to be present only in eastern Africa, at low frequencies.

In this study we analyzed more than 1,600 Y chromosomes from 55 African populations, using both new and previously described biallelic markers, in order to refine the phylogeny and the geographic distribution of the E3a haplogroup.

The most common E-DYS271 sub-clades (E-DYS271\*, E-M191, E-U209) showed a non uniform distribution across sub-Saharan Africa. Most of the E-DYS271 chromosomes found in northern and western Africa belong to the paragroup E-DYS271\*, which is rare in central and southern Africa. In these latter regions, haplogroups E-M191 and E-U209 show similar frequency distributions and coalescence ages (13 and 11 kyr, respectively), suggesting their involvement in the same migratory event/s.

By the use of two new phylogenetically equivalent markers (V38 and V89), the earlier tripartite structure of E3 haplogroup was resolved in favor of a common ancestor for haplogroups E-DYS271 (formerly E3a) and E-M329 (formerly E3c). The new topology of the E3 haplogroup is suggestive of a relatively recent eastern African origin for the majority of the chromosomes presently found in sub-Saharan Africa.

**P07.135****Genetic variability of Madeira archipelago inferred from Y chromosome, mtDNA and HLA system****A. C. N. Lemos, H. Spinola, R. Gonçalves, A. Fernandes, A. Brehm;***Human Genetic Laboratory, Funchal, Portugal.*

The Madeira Archipelago is composed by two inhabited islands, Madeira and Porto Santo. The first settlers of these islands came from north and south of Portugal and Europe (Flandres, France and Italy), jointly with some African slaves.

Three geographic groups were defined within the Archipelago: Funchal (FX), Southwest (SW) and Northeast (NE - including Porto Santo). The Y chromosome haplogroup followed the *Y Chromosome Consortium* and comparison with both mtDNA and HLA-A, HLA-B and HLA-DRB1 genes was performed. *Arlequin* was used to compare the three geographic groups within the archipelago and to calculate genetic diversity of Y chromosome SNPs, mtDNA and HLA systems between and within each group.

No significant haplotypic differences were found regarding the Y chromosome SNPs for these three groups, opposite to mtDNA and HLA systems encountered between Southwest and Funchal. We also found major European influence although some African traces are present. The highest level of genetic diversity was found in Funchal for

both mtDNA and HLAs.

The aim of this study was to determine the genetic background of the Madeira population, to search for differences within the Archipelago and find out the influence of the colonization in the actual genetic structure of this population.

**P07.136****Prehistoric migrations out of East Europe: phylogeography of Y-chromosome haplogroups N2 and N3a****V. Kharkov<sup>1</sup>, O. Medvedeva<sup>2</sup>, K. Khamina<sup>2</sup>, V. Stepanov<sup>1</sup>;**<sup>1</sup>Institute for Medical Genetics, Siberian Branch of Russian Academy of Medical Sciences, Tomsk, Russian Federation, <sup>2</sup>Tomsk State University, Tomsk, Russian Federation.

To reveal the structure and phylogeography of N2 and N3a Y-chromosomal haplogroups and to reconstruct their origin, the analysis of YSTR-haplotypes was carried out using seven YSTR loci of NRY (DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS394 (DYS19)). A total of 234 samples belonging to N2 and 903 belonging to N3a from different ethnic population samplings of Europe, Siberia, Central Asia and Far East were analyzed. The results of the analysis evidenced a higher genetic diversity for N2 and N3a haplogroups in European populations as compared with Asian ones. Median networks showed the occurrence of different haplotype clusters for Europeans and Asians. Age of STR variation for N3a haplogroup was 14.2 thousand years (12.3 only for Europe and 8.6 for Siberia) and for N2 haplogroup it was 12.6. A high frequency of N2 and N3a haplogroups in some Siberian populations is the consequence of strong founder effect events, the age of which reached 3-5.5 thousand years. Pairwise values of Fst showed that, in Siberia, neighboring populations were characterized by a higher level of genetic differentiation than European ones. Cluster analysis also revealed relative proximity of European populations to each other as compared to Asian populations. These results suggest that an isolation of the regional group of populations occurred soon after the origin of the N2 and N3a haplogroups. Evenks and Yakuts displayed highly specific overlapping N3a haplotype spectra, atypical for other Siberian ethnic groups. Thus Eastern Europe is the most probable place of N2 and N3a haplogroups origin.

**P07.137****Y-chromosome lineages in Xhosa and Zulu Bantu speaking populations****R. P. A. Gonçalves, H. Spínola, A. Brehm;***Human Genetics Laboratory, Funchal, Portugal.*

Y-chromosome Single Nucleotide Polymorphisms have been analysed in Zulu and Xhosa, two southern Africa Bantu speaking populations. These two ethnic groups have their origin on the farmer's Bantu expansion from Niger-Congo border towards sub-Sahel regions on the southern tip of the continent, during the past 3000 years.

Seven different Y-chromosome haplogroups were found in Zulu contrasting with only two in Xhosa. E3a, a common haplogroup among West sub-Saharan associated to Bantu migration was the most prevalent in both populations (56.9% in Zulu and 90% in Xhosa). The second most common haplogroup was E2 (29.3% in Zulu and 10% in Xhosa), present both in West and East African populations.

The present-day Zulu and Xhosa paternal legacy is essentially of West sub-Saharan origin. Zulu population shows a most diverse genetic influence comparing to Xhosa, revealing some pre-Bantu expansion markers and East African influences. Zulu presents 8.6% Y-chromosome haplogroups (A, B, J1) of non-Bantu influence that could indicate gene flow from other populations, particularly Khoisan.

**P07.138****Y-chromosome lineages and kinship relation in Central Eastern Sardinia****P. Rizzu<sup>1</sup>, L. M. Pardo<sup>1</sup>, G. Piras<sup>2</sup>, K. J. van der Gaag<sup>3</sup>, D. Sonderman<sup>1</sup>, M.****Monne<sup>2</sup>, A. Gabbas<sup>2</sup>, N. Bradman<sup>4</sup>, P. de Knijff<sup>3</sup>, A. Ruiz-Linares<sup>4</sup>, P. Heutink<sup>1</sup>;**<sup>1</sup>Department of Clinical Genetics, Section Medical Genomics, Amsterdam, The Netherlands, <sup>2</sup>Biomolecular and Cytogenetic Center, Dept. of Hematology and Oncology, San Francesco Hospital, Nuoro, Italy, <sup>3</sup>Forensic Laboratory for DNA Research, Leiden University, Leiden, The Netherlands, <sup>4</sup>The Galton Laboratory, Department of Biology, University College London, London, United Kingdom.

Genetic isolates have been successfully used in the study of complex traits, mainly because they allow a reduction in the complexity

of the genetic models underlying the trait. Prior knowledge of the genetic structure of the isolate is therefore a fundamental prerequisite for designing and carrying out successful association studies of complex disorders.

Sardinians has long been the object of study by geneticists by virtue of their ancient origin and long-standing isolation. Some studies suggest that the Sardinians are a relatively homogenous population with no significant heterogeneity among sub-regions. These reports are however in contradiction with several others demonstrating the existence of differentiated sub-regions, molded by natural, cultural barriers and historical events.

Our aim is to determine the extent of homogeneity in the Central-Eastern Sardinia that includes the archaic area as defined by archeological, linguistics and genetic studies. We determined Y-chromosome lineages in 256 unrelated Sardinian males from this area using a panel of informative biallelic markers (SNPs) and microsatellite (STRs). In addition to sex-specific markers we also used autosomal SNPs (500K Affymetrix chips) in 100 of the DNA samples to determine accurately kinship values.

Our analysis shows that the frequency of the major Y haplogroups clearly sets this population apart from the rest of the Europeans haplogroups.

Our results allow to evaluate how past peopling and demographic events might impact genome wide association study design for complex disorders that show a high incidence or a founder effect in this part of the island such as Diabetes type-1 and Breast-cancer.

#### P07.139

##### Caracterization of the mitochondrial haplogroups of two Andean populations (Aymaras and Quechuas) from the Bolivian Altiplano: Comparison to other South-American populations

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Mitochondrial DNA has been widely used in human population genetic studies. It has been used to treat the colonization process of the New World. Particularly, in South-America, the Andean region with its geographic, environmental and historical particularities is an important area for these studies.

We have analyzed the mitochondrial DNA diversity of two Amerindian populations from the Andean Altiplano belonging to the two main Amerindian linguistic groups in Bolivia, namely Aymaras and Quechuas. The Aymara population is situated between La Paz and the Titicaca Lake and the Quechua population located in the Potosí department. Our aim is to provide new mtDNA data from these two Andean Altiplano populations.

Haplogroup (A, B, C, and D) and sub-haplogroup determinations have been carried out through RFLP analysis in the coding region, as well as through DNA sequencing of the HV I and HV II regions (16020-250) in 190 non-related individuals.

After the determinations, the allele frequencies have been calculated and compared to other South-American populations for which data are available in the literature. Statistical analyses have been carried out in order to assess the genetic relationships between just the two populations of this study and also regarding three geographical levels: South-America, Central Andes and Bolivia.

#### P08. Genomics, technology, bioinformatics

##### P08.01

##### DECIPHER (DatabasE of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources) - <http://decipher.sanger.ac.uk>

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DECIPHER is a web-based, interactive database which provides a link between phenotype and chromosomal rearrangement utilising the En-

sembl genome browser and other bioinformatics resources.

DECIPHER provides the architecture for international collaborative effort identifying new syndromes and genes involved in human development and disease, for interpreting array CGH data and improving medical care for patients with congenital abnormalities. The DECIPHER Consortium has grown considerably over the last four years with database entries of over 1500 patients from approximately 90 centres worldwide.

In DECIPHER, molecularly defined rearrangements (e.g. from array-CGH) are mapped on to the reference sequence for viewing in Ensembl. Genes within the affected region are identified and prioritised according to their relevance to the phenotype. Clusters of rearrangements within the same region in patients with comparable phenotypes enable new syndromes to be defined and published.

Other features in DECIPHER which aid in the interpretation of microarray data include:

Trio analysis tool - A trio of an affected individual and parents are analysed to determine de novo or familial/inherited conditions.

Gene prioritisation tool - advanced text mining searches PubMed for associations between highlighted genes and phenotypes.

Search tool - a search engine for 'consented' data within DECIPHER to facilitate the identification of rearrangement clusters and links between phenotype and genomic location.

DECIPHER enables international collaborative research on developmental disorders and provides a powerful knowledge base for clinical diagnosis and management of patients with congenital abnormalities.

##### P08.02

##### 3C on FOXL2.

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Defective long distance gene regulation is an emerging mechanism underlying human disease. Blepharophimosis syndrome (BPES), an autosomal dominant condition affecting eyelid and ovary development, is caused by mutations in the *FOXL2* gene. Its expression is strictly regulated, which was illustrated by the recent identification of deletions upstream and downstream of its transcription unit as underlying cause of BPES. We demonstrated that these rearrangements remove several conserved non-coding sequences (CNCs) harbouring potential long-range *cis*-regulatory elements.

Here, we used Chromosome Conformation Capture (3C) to identify long-range interactions of *cis*-regulatory elements with the *FOXL2* promoter in two adult *FOXL2* expressing cellular systems. We found that in adult ovarian granulosa cells and fibroblasts three long-range *cis*-regulatory sequences located 177 kb, 283 kb and 360 kb upstream of *FOXL2* come in close vicinity to the *FOXL2* core promoter. Noteworthy, 3C in human fibroblasts derived from a BPES patient with a heterozygous deletion of the region encompassing the regulatory element at 283 kb, revealed decrease of interaction of the deleted element and the *FOXL2* core promoter and the two other regulatory elements. Interestingly, the element at 283 kb corresponds to a sequence deleted in the Polled Intersex (PIS) goat, which is an animal model for BPES. In conclusion, we hypothesize that the interaction between the *cis*-regulatory element located at 283 kb and the *FOXL2* core promoter is essential to establish efficient transcriptional regulation of *FOXL2* expression.

##### P08.03

##### Study of the antisense transcript to AFAP1 human gene

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Antisense regulation of gene expression is a widespread but not well-understood mechanism of gene expression regulation. Recently we have carried out a whole genome *in silico* search of *cis*-antisense

clusters of transcripts in humans. The developed database revealed a significant number of sense—antisense pairs consisting of one EST cluster expressed predominantly in normal tissues and another cluster with tumor-specific expression. The role of antisense transcripts in the regulation of oncogenes and tumor suppressor genes warrants functional research. Here we describe and characterize an antisense mRNA asAFAP overlapping human *AFAP1* gene.

*AFAP1* encodes for an actin filament binding protein, which serves as a modulator of actin filament structure and integrity. It also is able to relay a signal from receptor tyrosine kinases through PKC $\alpha$  to Src protein kinase. It has been shown that *AFAP1* is overexpressed in prostate cancer and contributes to tumorigenic growth. We hypothesized that the transcription of asAFAP antisense mRNA may lead to suppression of sense *AFAP1* gene expression and a compensatory restrain added to one of the mitogenic signaling pathway that is unlikely to be supported by natural selection in the tumor cell population. To study the intriguing phenomenon of tumor-specific asAFAP antisense expression we performed detailed *in silico* analysis of asAFAP sequences and experimentally quantified this transcript in normal and tumor human tissues.

We have specified the exon-intronic structure of asAFAP antisense transcript and carried out the expression analysis of *AFAP1* sense—antisense pair in normal and tumor human tissues.

#### P08.04

#### High-resolution breakpoint mapping of human chromosome 21 segmental aneuploidies for genotype-phenotype correlation and identification of underlying genomic architecture

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As part of the European FP6-sponsored AnEuploidy consortium we are involved with work package 2: Genotype-phenotype correlations in human aneuploidies. The objective of this work package is to study the phenotypic consequences of gene dosage imbalance in the human population.

One of our roles in this project is the characterization of segmental aneuploidies of HSA 21. Until now we collected 19 cases with detailed clinical information, enabled by using a standardized phenotypic list. Cell lines and/or DNA are available for all patients. Karyotyping as well as additional analysis (FISH, microarray-based high-resolution genome profiling) were performed for the majority of cases. We have used a chromosome 21 specific oligo-array with 385,000 oligonucleotides to further delineate the genomic rearrangements in this cohort. Breakpoint fine mapping of approximately 1kb accuracy has been performed for the most cases, enabling breakpoint sequencing as a next step. For one case with a partial chromosome 21 deletion (46,XY,del(21)(q11.2q21.3)) the rearrangement coincide with bordering segmental duplications (SDs) that have identical orientation and high (>95%) similarity. This suggests that recurring deletions and/or corresponding duplications of similar size that are mediated by NAHR (non-allelic homologous recombination) may exist. These analysis of the underlying genomic-architecture are enabled by in-house developed software tools.

Detailed genotype-phenotype correlations are ongoing for all patients to get a better insight into the underlying gene dosage imbalances. Expansion of the patient cohort and transcriptome analysis are planned as joined efforts of the consortium.

#### P08.05

#### Artificial intelligence applied in finger prints identification

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In this paper we propose a new database search method with results in finding the most nearest existing data. The searching method is based on the artificial intelligence concepts named Genetic Algorithms combined with polynomial approximation.

In the first stage of the process for each record in the database we create an algebraic polynomial named characteristic function and we update the database with the polynominal coefficients.

For the second stage we process the input data also to create an algebraic polynomial approximation. This method was used in our previous research in numerical approximation with Genetic Algorithms.

In the last stage we search all the polynomials created in the first stage which approximate the minimum requirement of the polynominal representing the input data.

If the difference doesn't satisfy the minimal requirement, we consider this data inexistent in the original database and we could save this data like a new record.

We applied this method to interpret the 2d coordinates of the points which represent the particularities of the finger prints.

#### P08.06

#### Genomic profiling to identify novel genetic risk factors for Behcet's disease

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Introduction: Behcet's disease (BD) is a multisystemic immuno-inflammatory disorder characterized by a generalized vasculitis, particularly at oral and genital mucosa and eye (uveitis). Although there is evidence for environmental risk factors, epidemiological and family studies strongly support the existence of genetic risk factors for BD. The only established genetic predisposition for BD is the HLA-B\*51 allele on chr. 6p21, which explains only 19% of its overall genetic susceptibility. Furthermore, a recent linkage study on Turkish families found strong evidence for linkage with BD at 6p22-24 and 12p12-13. Case-control association studies on biological candidate genes have so far mostly been inconclusive. To identify new susceptibility genes for BD, we are conducting the "genomic convergence" approach, which combines data from whole genome linkage screens with expression studies to determine which genes will be tested in association studies.

Methods & Materials: We performed gene expression profiling in peripheral blood mononuclear cells of carefully matched cases and controls using Affymetrix GeneChip Human Genome U133 Plus 2.0 microarrays.

Results: Preliminary analyses identified 131 genes differentially expressed among 11 cases and 11 controls (1.2 fold-change cutoff and p-value<0.05), 18 of which map to the linkage peaks in Turkish families.

Conclusions: These 18 genes differentially expressed among BD patients and controls constitute excellent candidates genes for association studies in this disease. We are currently confirming the expression studies in a larger dataset.

#### P08.07

#### The centralized DNA-extraction, quality control, storage and sample logistics center for EU-project Genetics of Healthy Aging

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The National Public Health Institute of Finland (KTL), Department of Molecular Medicine (MLO) has over the past decade developed an infrastructure for handling an extensive number of biological samples collected in large epidemiological and population based studies ([www.nationalbiobanks.fi](http://www.nationalbiobanks.fi)). The biobank consists of centralized DNA-extraction, quality control, storage and sample logistics and has developed a database and sample logistics system applicable to any biobank handling DNA samples. The center is equipped with state of the art bar coding system, automated DNA extraction equipments, liquid handling robots, storage facilities and tailor made data management tools. The biobank has served as a DNA logistics center for several research projects, including the EU funded project; Genetics of Healthy Aging (GEHA) ([www.geha.unibo.it](http://www.geha.unibo.it)).

The aim of the GEHA consortium is to collect DNA samples from 2650 long-lived 90+ sib pairs and controls from 15 European countries. The samples are shipped to Helsinki, DNA is extracted, stored, quality checked and distributed to the genotyping laboratory CEPH in France. By the end of 2007, 9813 samples from 6867 individuals had been extracted. The method of choice for nearly all of the GEHA samples has been the automated extraction equipment Autopure LS. The failure rate of extraction has been very low < 1%. Average yield for blood samples is 31,4 $\mu$ g/ml of blood and 1,8 $\mu$ g for cheek swab samples.

From all the subjects 4745 samples have been tested for PCR-functionality and monitored in case of sample mix-ups or contamination. During QC only 0,8% of the samples had to be excluded.

#### P08.08

#### Using the bioinformatic tools to choose the SNPs with highly possible phenotypic effect

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Common polymorphisms, such as SNPs, in human gene promoters are the significant factors influencing differential gene expression underlying natural phenotypic variation. A number of bioinformatic tools were developed recently, which are useful in prediction of "on its own" functionally important SNP in promoters utilizing the knowledge about transcription factors binding the DNA. We used such the resources for a pilot search of functional SNPs in seven immune response modifying genes: STAT1, IL10, IL12B, IFNG, IFNLR1, MCP-1, TLR-2. Firstly, all 61 the SNPs were chosen in the promoters of these genes using dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>), and Ensembl (<http://www.ensembl.org>). Then, the selection of a minimal sufficient number of SNPs was done using SNPselector (<http://www.snpselector.Duhs.duke.edu>) and PupaSNPFinder (<http://www.pupasnp.org>). Finally, Genomatix MathInspector (<http://www.genomatix.de/mathinspector.html>) was utilised to predict a possible transcription factors (TFs) binding efficiency change due to the SNPs chosen. Eighteen SNPs in promoter region of seven genes were analyzed by MathInspector and it was found that the nucleotide substitution in seven SNPs caused new binding sites for TFs. The potential functionality of these SNPs is under current experimental validation in our group. Thus, bioinformatic approaches to the analysis of gene promoters allows reducing the search space for candidate SNPs and focusing on the SNPs with specific characteristics. Such *in silico* analysis facilitate understanding of specific features of gene promoters under the study and provide information on the genes functional variability.

#### P08.09

#### Breast cancer diagnostics: CSCE screening using the BioNumerics® software.

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INTRODUCTION. CSCE technology can be used for indirect mutation scanning (e.g. BRCA1/2 mutation detection). The method is sensitive and more rapid than full gene sequencing and is therefore time and cost saving. By the use of multi-capillary sequencers, high throughput routine screening becomes feasible, but requires the availability of reliable automatic mutation detection software.

DATA ANALYSIS is performed directly on the ABI .FSA files. Files are imported in batches through the use of a BioNumerics® plugin, a script based dynamic extension of the software that uses a file naming strategy to automatically import up to 200 traces with up to 20 samples each. BioNumerics® provides an adapted database environment to store all imported data and takes care of all data management activities. The plugin offers a proper analysis tool for the mutation detection: Peak matching is done using a proprietary algorithm that uses five user-adjustable curve parameters allowing to compare normalized peak shapes. The result is a fast, sensitive and reliable peak matching that can be used to discriminate typical 'wild type samples' from 'heterozygous mutants'. For each target PCR product, one or more target variants can be defined, allowing the creation of polymorphic variants. The result is displayed in a clear overview report with color indication of reference peaks, positive matches, mismatches, failed peaks and problem cases for which verification is required. For the latter click and zoom functions are available to quickly evaluate all matching parameters on the screen.

#### P08.10

#### High Quality Mutation Detection

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Accurate mutation calling and quality data have been identified as key components of direct sequencing by many clinical researchers. We used a new bioinformatics software, Variant Reporter™ to detect muta-

tions in large volume of data sets. This analysis tool provides improved algorithm for SNP detection that are trained to discover accurate sequence variations and report review status for traceability. It helps to create expressive Quality Control Data reports for large data sets and create annotated projects that contain trace files and data annotation. Data sharing abilities between users, such as between a bioinformatics team and end users, will be demonstrated. The guided workflow gives a new or advanced user confidence in a short period of time. In this poster we will highlight how core team can use the new Quality Control metrics and how end users can share the accurate results.

#### P08.11

#### Prevalence of mutations in Troponin T (TNNT2) and Troponin I (TNNI3) in Czech hypertrophic cardiomyopathy (HCMP) patients.

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Hypertrophic cardiomyopathy (HCM) is a serious cardiovascular disease with autosomal dominant inheritance, caused by mutation of genes coding for structural or regulation proteins of sarcomeres of the heart muscle. Troponin T (TNNT2) and Troponin I (TNNI3) are important part of sarcomere of heart muscle and mutations in their genes are responsible for development of HCM.

We have performed complete sequencing of TNNT2 and TNNI3 genes in 100 HCMP patients, previously diagnosed by Electrocardiography. We have recorded a total of 4 positives. Of the different mutation types detected, there was 1 novel mutation, which, to date, was not recorded in any of the major HCMP databases. A wide variability of the mutation malignancy was recorded with respect to the disease manifestation for different mutation types.

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#### P08.12

#### Disentangling molecular relationships with a causal inference test

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There has been intense effort over the past couple of decades to identify loci underlying quantitative traits as a key step in the process of elucidating the etiology of complex diseases. However, a stumbling block has been the difficult question of how to leverage this information to identify molecular mechanisms that explain quantitative trait loci (QTL). We have developed a formal statistical test to quantify the strength of a causal inference pertaining to a measured factor, e.g., a molecular species, which potentially mediates the causal association between a locus and a quantitative trait. We applied the test to infer causal relationships between transcript abundances and obesity traits in mice and also to reconstruct transcriptional regulatory networks in yeast. We treat the causal inference as a 'chain' of mathematical conditions that must be satisfied to conclude that the potential mediator is causal for the trait, where the inference is only as good as the weakest link in the chain. This perspective naturally leads to the Intersection-Union Test framework in which a series of statistical tests are combined to form an omnibus test. Using computer simulated mouse crosses, we show that type I error is low under a variety of non-causal null models. We show that power under a simple causal model is comparable to other model selection techniques as well as Bayesian network reconstruction methods. We further show empirically that this method compares favorably to TRIGGER for reconstructing transcriptional regulatory networks in yeast, recovering 6 out of 8 known regulators.

#### P08.13

#### Identification of a potential regulatory element forming a hairpin structure within the 3'UTR of CDK5R1

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CDK5R1 encodes for p35, a regulatory subunit of CDK5 kinase, which is fundamental for normal neural development and function. CDK5R1

has been implicated in neurodegenerative disorders and proposed as a candidate gene for mental retardation. The remarkable size of CDK5R1 3'UTR, which is highly conserved in mammals and contains AREs and miRNA target sites, suggests a role in post-transcriptional regulation of its expression. The insertion of CDK5R1 3'UTR into luciferase gene causes a decreased luciferase activity in four transfected cell lines. A 3'UTR region (named C2) leads to a very strong luciferase mRNA reduction, owing to a significantly lower half-life, indicating accelerated mRNA degradation. This fragment was dissected into smaller regions and a 65 bp sequence (C2.11), in which no known regulatory elements were predicted, has been identified to be responsible of the decreased gene expression. A stable structural motif (forming a hairpin) of C2.11 fragment was predicted by RnaProfile and SFold programs, both starting from the isolated fragment and considering it within the whole 3'UTR. Lowering of luciferase levels for the construct with an intact hairpin structure in contrast with unchanged levels for four mutated/deleted structures suggests that this putative element might really have a regulatory role. Since complementary mutations restoring the hairpin did not affect luciferase activity, we suggest that both the sequence and the structure are essential for the ability of C2.11 fragment to reduce transcript stability. Further mutagenesis experiments, binding assays and RNase protection assays will disclose the function of this novel CDK5R1 3'UTR regulatory element.

#### P08.14

#### Computational analysis of structural and non-structural proteins of chikungunya virus - mosquito borne disease as potential target for vaccine

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Chikungunya virus (CHIK), an alphavirus borne by *Aedes* mosquitoes that produces dengue-like illness in humans, characterized by fever, rash, painful arthralgia, and sometimes arthritis throughout sub-Saharan Africa, Southeast Asia, India, and Western Pacific. Recent widespread geographic distribution, recurrent epidemics, and infection of military personnel, travelers and laboratory staff working with CHIK have indicated need for more understanding and to have safe and efficacious vaccine. In our present study we have analyzed the characteristics of structural and non-structural proteins synthesized by CHIK using computational tools and predicted the potential vaccine candidates. CHIK contains two proteins - non-structural and structural. Computational analysis of non-structural protein revealed that it is 275.65 kDa hydrophilic protein, pI 6.841, while that of the structural protein revealed a 138.88 kDa hydrophilic protein of pI 8.88. Antigenic prediction sites on non-structural and structural proteins predicted were examined for their use as vaccine candidates for effective control of the disease. Positions of alpha helix and b-sheets in the secondary structure of the proteins were predicted which laid the path for 3D structural characterization of the target proteins. On analyzing hydrophathy plot, structural protein and non-structural protein were found to be hydrophilic. Using nucleotide sequences of the proteins, degenerate primers were designed for its use in PCR based diagnostic identification of the CHIK. Primers designed could find its use as a diagnostic tool for identifying CHIK infected patients specifically. The predicted antigenic sites could be used as potential vaccine candidates.

#### P08.15

#### CHDWiki: a comprehensive tool to gather and manage cardiogenetic data

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We present a Wiki based information system designed for the collaborative annotation of genes involved in congenital heart defects (CHD). In this context, a Wiki has many appealing features such as collaborative user-friendly publication. However, a major obstacle for its use is that, unlike databases, it lacks structure and semantics, which prevents the use of further computational knowledge discovery approaches. To benefit from both the Wiki flexibility and the databasing advan-

tages, we extended the MediaWiki platform to allow the inclusion and the interaction with external data and programs. The current online project contains: (i) genes and gene mutations associated to CHDs (local curated database), (ii) links from CHD genes to related patient case reports (local cytogenetic database), (iii) links from CHD genes to publicly available descriptions of copy number variants, (iv) an interactive view of this information on chromosomes. Moreover, CHD candidate genes can be prioritized (*Endeavour*) based on easily selectable training genes associated to CHD types. CHDWiki promises to be the central resource/reference for CHD genetics. It can be viewed as a dynamic review of all the knowledge published in this field. Additionally, the Wiki goes further by managing information on promising candidate genes. The CHDWiki will be the most comprehensive resource available on genes associated to CHDs with 48 genes, 40 CHDs and 135 manually curated associations. While initially dedicated to this concrete project, the system is generic and allows the rapid development of Wikis augmented by structured data and analysis results.

<sup>1</sup>Aerts et al., Nat Biotech, 2006;24:537

#### P08.16

#### Copy number variants and gene expression in the mouse

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Copy number variants (CNVs), defined as large stretches of DNA that vary in number of copies among phenotypically normal individuals, are a source of phenotypic variation. To achieve a more complete mapping of mouse CNVs, we used whole-genome oligonucleotide array comparative genome hybridization to identify CNVs in 13 inbred strains and in 21 wild mice. Using a hidden Markov model, we predicted some 3800 CNV candidate regions, which we subsequently validated using a custom-made array. Thus multiplying by more than three the number of copy-number variable regions previously reported in the mouse genome.

To address whether CNVs affect gene expression, we assessed the expression levels of 45'037 transcription units in liver, kidney, brain, heart, lung and testis of six inbred mouse strains. We found that the variance of the expression levels for each of the recorded tissues is significantly larger for genes mapping inside than for genes mapping outside of CNVs, suggesting that copy number variation affects the variability of gene expression and must be taken into account when considering phenotypic differences between strains. Similarly, the genes mapping on the flanks of the CNVs, despite their not varying in copy numbers, display a modification of their relative expression levels. This phenomenon is effective over several hundreds of kilobases away from a breakpoint. It is present in all assessed tissues and persistent throughout mouse development. Thus our results demonstrate that changes in genome structure influence not only gene dosage but also the expression of neighboring genes.

#### P08.17

#### Development of TaqMan® Copy Number Assays for Copy Number Analysis

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Copy number variation is an important polymorphism in the human genome that can be associated with genomic disorders or various diseases. Accurate detection of copy number differences is critical for understanding how copy number variation may play roles in diseases such as cancer, immune diseases, and neurological disorders, and also drug response. Although array-based technologies are powerful for genome-wide CNV discoveries and micro-deletion/micro-duplication syndrome studies, more quantitative technologies with high accuracy, specificity, and sample throughput are necessary to validate identified copy number changes and to detect deletions and duplications for large sample sizes in candidate regions or genes.

Here we report our progress on the development of TaqMan Copy Number Assays. High quality targets for assay design are selected across the whole human genome. A proprietary assay design pipe-

line generates assays for genomic DNA targets, and includes genome specificity checks and screens to minimize oligo interactions. TaqMan Copy Number Assays are run together with a reference assay and gDNA in a duplex real-time PCR. The TaqMan Copy Number Assay detects the target gene or genomic sequence of interest and the reference assay detects a sequence that is known to be present in two copies in a diploid genome. Relative quantification is achieved using a data analysis tool which has been developed for copy number determination. We will discuss progress in development of the assay design pipeline, and data analysis tool, and assay development including studies of reference assays, gDNA input titration, and the accuracy and precision of TaqMan CN assays.

#### P08.18

##### In silico search for the cryptic RSS in repetitive elements of human genome

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Cryptic recombination signal sequences (cRSS) are targets sites of RAG1/2 proteins when the V(D)J-recombination system gets out of control. Early, we showed that in the human genome (outside the Ig and TCR loci) there are 5649 cRSS which supposedly have high recombination potential (their heptamers and nonamers corresponded to CACAGTG and ACAAAAACC sequences or differed from them for 1-2 not functional nucleotides). Some of them can hypothetically participate in the formation of intragenic deletions and inversions. At present, having matched coordinates of such cRSS with coordinates of 9933066 known in the human genome repetitive elements in silico, we observed that 3500 (61 %), 265 (5 %), 102 (2 %), 51 (1 %) 7 (0,1 %) cRSS are the structural elements of non-LTR retrotransposons (AluY, AluSx, L1, MIRb, etc), endogenous retroviruses and LTR retrotransposons (LTR1B, MLT1C, ERVL-E, etc), DNA transposons (Charlie1, MER58A, Tigger1, etc), simple repeats ((CA)n, (CAAA)n, etc) and other repeats (not identified) respectively. Having researched the localization of such cRSS, we observed that in 85 % cases the nucleotide sequences of motives are fully localized inside repeats and in 15 % cases are localized partly. In whole, cRSS were found in structure of 414 unique types of repeats of 16 families. We consider, that many types of repetitive elements can participate in spreading of motives which can theoretically mediate instability in human genome when the V(D)J-recombination system gets out of control.

#### P08.19

##### Development of a TaqMan® Cytochrome P450 Panel Array and Positive Controls

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The Cytochrome P450 enzymes: CYP2D6, CYP2C9 and CYP2C19 metabolize the majority of currently prescribed drugs. Pharmacogenetic analysis of polymorphic alleles of their genes has demonstrated numerous links between functional polymorphisms, having absent or altered enzyme activity, and cases of non-response or adverse drug reactions. Genotyping these variants can have a significant role in predictive drug metabolism and safe, efficacious drug therapy.

Applied Biosystems offers over 2600 TaqMan® Drug Metabolism (DME) Genotyping Assays that detect polymorphisms in coding and regulatory regions of over 220 DME genes. To facilitate pharmacogenetic research, TaqMan® DME Assay panels on TaqMan® Arrays were developed including a P450 array containing 48 assays to important CYP2D6, CYP2C9 and CYP2C19 variants. Arrays are 384-well microfluidic cards that are pre-loaded with assays, greatly reducing experimental preparation time and eliminating the need for liquid-handling robots or multi-channel pipettes. Benchmark tests conducted to compare the performance of assays run on arrays to data from 384-well plates showed that assays on arrays can be genotyped with the same high success rate as on plates. An interactive data analysis tool, AutoCaller™ software, enabled overlaying and viewing cluster plots from multiple arrays and facilitated genotyping analysis. Synthetic positive control templates for each assay were run on arrays in pools to represent all 3 genotypes; controls demonstrated assay functionality for each probe and aided genotype calling. TaqMan® DME SNP

Arrays and positive control templates (available through Gene Link™) offers an easy to use platform for accurate, reliable genotyping of drug metabolism and response variation.

#### P08.20

##### GEN2PHEN: An international effort towards the universal databasing of gene-disease relationships

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With disease studies and genomics research producing ever larger datasets, there is an urgent need for advanced informatics solutions that can handle such extensive information. Launched in January 2008, the GEN2PHEN project (Genotype-To-Phenotype Databases: A Holistic Solution) aims to help address this need.

The GEN2PHEN consortium (<http://www.gen2phen.org/>) involves 19 research and company partners; including 17 from Europe, one from India, and one from South Africa. Funding of 12 Million Euro from the European Commission (7th Framework Programme) is bolstered by additional funds provided by the partner institutions. Being a key European program, GEN2PHEN is intimately connected with other major infrastructure projects such as BBMRI and ELEXIR.

The main objective of GEN2PHEN is to establish the technological building-blocks needed to evolve today's diverse databases into a seamless genotype-to-phenotype (G2P) biomedical knowledge environment, tied into genome browsers like Ensembl.

The project's specific objectives include:

- 1) Analysis of the current G2P informatics
  - 2) Analysis of ethical aspects that need to be addressed
  - 3) Development of key standards
  - 4) Creation of generic database components and integration solutions
  - 5) Creation of search modalities and data presentation solutions
  - 6) Facilitation of data flows into G2P databases
  - 7) Creation of a 'G2P Knowledge Centre' providing information exchange solutions, search/analysis tools, and support for primary data and comment deposition
  - 8) Deployment of GEN2PHEN solutions to the community
  - 9) Addressing questions of system durability and long-term financing
- The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 200754.

#### P08.21

##### Two-Dimensional Electrophoresis of Nucleic Acids to Assess Quality of Samples and Efficiency of Molecular Methods

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Two-dimensional (2D) electrophoresis allows separation of molecules based on different characteristics. Although widely used in proteome studies, 2D electrophoresis of nucleic acids has so far only had limited applications mostly in studies on DNA metabolism. We have invented several new techniques of 2D electrophoresis of nucleic acids. These techniques can be used to simultaneously assess length distribution and strandness i.e. amount of single-stranded DNA, double-stranded DNA (both A and B forms) and RNA-DNA hybrids in various samples. DNA lesions detected with our technology include nicks, double-stranded breaks, base modifications and drop outs leading to

compromised base pairing or even generation of single-stranded DNA molecules. Various lesions in DNA can happen *in vivo* and *in vitro*. We have observed extensive damage to DNA due to various types of lesions formed *in vitro* upon storage of biosamples and under conditions simulating various laboratory procedures. These lesions in the original samples influence the efficiency and quality of complex molecular procedures such as quantitative amplifications and random labelling reactions. 2D Strandness-Dependent Electrophoresis can also be used to assess efficiency of cDNA synthesis, a critical step in genomic expression studies. Typical yield of double-stranded DNA is again very variable reflecting the poor control of such a complex procedure. To make these techniques easily available to investigators, BioCule Inc. is marketing a dedicated automated instrument using 2D microgels.

#### P08.22

#### The acidic environment as essential attribute for the DNA functioning

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Experimental results were obtained that give the possibility to conjecture mechanisms by which the proteins (RNA polymerases) carry out promoter melting, which is important step in transcription process. We suppose that process of melting (opening of specific sites of ds-DNA) is induced by acting of acidic residues enriched regions of proteins. Experimental investigations of the DNA molecule in acidic environmental condition unambiguously have indicate that acidic area ( $\text{pH} < 5.0$ ), induces despiralization of DNA and process is reversible. The calorimetric measurements performed under different acidity condition have shown, that ds-DNA structural flexibility greatly depends on the value of solvent pH. It is shown that, by the increase of environmental acidity (in contrary to the alkaline environment), DNA structure undergoes considerable destabilization. We have demonstrated that if DNA destabilised under acidic treatment (for which stability of double helix considerably is reduced and most of its sites lack helical structure) is transferred in the neutral physiological environmental condition ( $\text{pH} 7.0$ ), DNA restores its nature helical structure throughout.

#### P08.23

#### The stochastic description of DNA hybridization

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There are a lot of works devoted to DNA hybridization problem, however practically in all works the process of hybridization is described deterministically. Such description is not full. The full description of DNA hybridization process should be done in the framework of stochastic model. For the stochastic description of DNA hybridization, let us introduce the conditional probability function, that there are a certain number of DNA duplexes on substrate at moment of time, provided that at a preceding moment of time on substrate there were another number of DNA duplexes. Let us consider DNA hybridization process as a discrete Markov process. We obtained equation for the change of conditional probability in time. Using this equation it is possible to calculate not only an average number of DNA duplexes, but also dispersion, correlation function and spectral density of DNA duplexes number on substrate. It is shown, that with raising the concentration of single-stranded DNA in solution the dispersion of DNA duplexes on substrate increases, and then passing through a maximum decreases, and at a very high concentration of single-stranded DNA the dispersion approaches zero. The results we have obtained can be used in DNA-chip technology. In many cases, the basic source of random variations of a signal during information read-out from DNA-chip is fluctuation of concentration of immobilized DNA, and this is result of chip production technology. Our calculations indicate that another source of fluctuation is present, which is connected to the stochastic nature of DNA hybridization process.

#### P08.24

#### Meta-analysis in the technology of DNA microarrays

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With the explosion of microarray technology, an enormous amount of data is being generated. Limited overlap among the published lists of discovered genes between similar studies makes it difficult to determine which, if any, exhibit truly effect of differential expression and prompting the question of whether microarray studies are inherently reliable. To address this issue it is of special interest to combine results across multiple studies of various research groups working on similar clinical entities. Systematic integration of gene expression data from different sources increases reliability of detecting differentially expressed genes. The challenge, however, is in designing and implementing efficient analytic methodologies for combining high-throughput genomic data from several related studies. We consider the meta-analysis of different microarray data sets using a fixed and random effect paradigm and demonstrate how relatively standard statistical approach yield promising results. We illustrated proposed method by integrating gene expression profiles from three different prenatal trisomy 21 studies based on Affymetrix microarray technology. We identified a novel set of 14 transcripts of potentially candidate genes, which are active in development of human embryo with trisomy 21 (Table 1). Our results show that this approach would serve as a plausible method for analyzing microarrays beyond the specific implications for trisomy 21.

Table 1. Statistically significant differentially expressed transcripts across studies.

Affymetrix ID	Gene Symbol	z - value	q - value
201086_x_at	SON	-5.380	0.002
200642_at	SOD1	-5.204	0.005
214988_s_at	SON	-4.952	0.005
202671_s_at	PDXK	-4.723	0.013
202217_at	C21orf33	-4.580	0.021
200944_s_at	HMGN1	-4.431	0.030
219767_s_at	CRYZL1	-4.428	0.030
201644_at	TSTA3	-4.292	0.049
202749_at	WRB	-4.246	0.049
218386_x_at	USP16	-4.262	0.049
200740_s_at	SUMO3	-4.204	0.049
213000_at	MORC3	-4.192	0.049
211065_x_at	PFKL	-4.185	0.049
216954_x_at	ATP5O	-4.165	0.049

#### P08.25

#### Multi-copy amplification of the exon 2 of the dystrophin gene in a Duchenne muscular dystrophy patient: A new approach by Oligo-Array-CGH

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Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD) are the most common inherited disorders of muscle.

Deletions are found in 55% of patients with BMD and 65% of patients with DMD. Point mutations account for ca. 30% of mutations, while duplications can be found in 5-10% of DMD patients.

MLPA (multiplex ligation-dependent probe amplification), is widely used as a reliable method to detect both deletions and duplications of dystrophin exons.

Here we describe a 5 years old male patients from lithuania with suspected Duchenne muscular dystrophy. He had a positive Gower sign and pseudohypertrophy of calf. Muscular biopsy showed muscular dystrophy with lipomatosis and fibrosis. CPK tests revealed a value of >22.000 U/L.

Analysis of the dystrophin gene by multiplex PCR excluded deletions. MLPA showed a single amplification of the exon 2 of the dystrophin gene. Surprisingly, calculation of the MLPA data revealed the presence of 4 to 5 copies of the exon 2. To validate this finding quantitative real-time PCR was performed giving also evidence for 4 to 5 copies of exon 2.

MLPA testing of the patients mothers demonstrated that the amplification was de novo.

To identify the exact range of the amplification a new approach by oligo-array-CGH was conceived. Shortly, a customer-designed 44k

Oligo-Array (Agilent) was created. Multiple probes on this array spanning the amplified region of exon 2 of the dystrophin gene. In a next step Array-CGH data will provide a basic for the design of PCR systems for exact determination of the amplification limits.

#### P08.26

#### Role of the kinase DYRK1A in cerebral cortex development: effects on the transcriptome

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DYRK1A is a dual-specificity protein kinase involved in brain development. Human DYRK1A is located in the Down Syndrome (DS) Critical Region of chromosome 21 and its overexpression has been associated to the neurological defects observed in DS. Nevertheless, DYRK1A function at the molecular level remains poorly understood. As DYRK1A substrates include several transcription factors, we have analyzed the impact of Dyrk1a dose reduction on the transcriptome of mouse cerebral cortex at postnatal day 0 (P0) and 7 (P7). To this end, global gene expression of Dyrk1a+/- and Dyrk1a+/+ cortices were compared using Affymetrix chips.

Microarray results revealed deep changes in gene expression extending into the set of 3169 genes analyzed. Among those, 22% and 5% showed different expression levels between genotypes at P0 and P7 respectively (adj-p<0.05). Despite this global perturbation, changes in the expression of individual genes were subtle: only 1% (at P0) and 3% (at P7) of the differentially expressed genes showed a fold-change >2. Gene Ontology analysis revealed enrichment in transporters within the down-regulated genes and in DNA binding molecules within the up-regulated genes both, at P0 and at P7. Among the genes deregulated between genotypes, 61 were common to both developmental stages. Interestingly, their regulation showed a similar trend at P0 and P7 but always opposite to the developmental trend (defined by the expression of such genes in Dyrk1a+/+ mice at P7 vs P0).

These results suggest that DYRK1A plays a pivotal role in cerebral cortex development acting on the regulation of the transcriptome.

#### P08.27

#### Re-examining the human genome

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Recent improvements in sequencing technologies enable the routine sequencing of large complex genomes. However the annotation of such genomes is still complex and there is no higher eukaryote that has all its coding sequences predicted to 100% accuracy. The HAVANA group (<http://www.sanger.ac.uk/HGP/havana/>) at the Wellcome Trust Sanger Institute is involved in manually annotating all the coding transcripts within the human genome through various international collaborations. The team is involved in the scale up of the ENCODE pilot gene annotation project (GENCODE) to the whole human genome. The project includes seven other partner institutes and will integrate computational approaches, expert manual annotation and targeted experimental approaches to generate a reference gene set. This will include analysis of pseudogenes, experimental validation of putative/novel genes and examination of the protein-coding potential of genes using comparative and structural analysis.

In addition, the Havana group collaborates with RefSeq at NCBI, UCSC and Ensembl to produce a core set of human and mouse coding genes for the Consensus CDS (CCDS) project. Any CCDS candidate transcripts where there is disagreement between WTSI/EBI and NCBI annotation are manually re-inspected, discussed and, where possible, an accord is reached on a structure (18293 human CCDS agreed to date). The end result is a combined, non-redundant gene set agreed by several of the major genome centers.

All annotation is displayed on the Vertebrate Genome Annotation (VEGA) database, a central repository for high quality manual annotation of finished vertebrate genome sequence with a three monthly release cycle (<http://vega.sanger.ac.uk/index.html>).

#### P08.28

#### An *in vivo* unbiased screen for enhancer activity using lentivector-mediated transgenesis

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Finding sequences that control spatial and temporal expression of genes is important to understand genome function. Here, we present an *in vivo* screen for enhancers in a contiguous 200-kilobase DNA fragment using lentivector-mediated transgenesis. Previous studies have used evolutionary conservation as an indicator of regulatory potential, but increasing evidence suggests that this criterion systematically overlooks functional sequences. We thus designed our study without any bias towards a particular sequence feature. We chose a mouse BAC corresponding to a region of Hsa21 because it contains the olig1 and olig2 genes that are expressed specifically in the CNS. In order to screen this fragment systematically for enhancer activity, we generated a library of 121 overlapping clones (sizes: 2-4kb) in a LacZ reporter lentiviral construct containing a minimal promoter. We generated lentivectors individually for each segment and injected them in pools of 10 or 20 in mouse oocytes. LacZ staining was performed on E11 embryos to identify expression patterns. The first six pools tested yielded 60 of 242 LacZ positive embryos with ~2.5 transgenes per embryo. To date, 7 fragments of 52 assessed were identified that potentially contain gene expression regulators. To confirm regulatory activity, 4 sequences were re-injected individually, 2 (one evolutionary conserved) of which were confirmed as tissue specific enhancers with stainings in the spinal chord and trigeminal ganglion compatible with olig expression. The method could be scaled up to cover large chromosomal regions, and determine what fraction of the constrained and non-constrained genome has regulatory potential.

#### P08.29

#### Enhanced workflow for sequencing PCR products by capillary electrophoresis

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Since the introduction of Sanger dideoxy sequencing, significant efforts have been directed toward increasing throughput by streamlining workflow. We describe here further enhancements for a PCR product resequencing workflow capable of reducing the total time, from beginning PCR reactions through completion of basecalling, to 6 hours or less. The workflow shown employs a new AmpliTaq Gold® Fast PCR Master Mix in conjunction with modified thermal cycler conditions to substantially reduce the time required for PCR amplification. Process time is further reduced through optimization of cycle sequencing (BDT v1.1) conditions. We have coupled these improvements with an efficient sequencing reaction cleanup protocol and decreased CE run time using the fast plates on a 3130xL. Overall data quality compares favorably with data obtained using previously documented methods. The increased efficiency and generation of high quality results is vital to both clinical and research applications in reducing time to discovery.

#### P08.30

#### Analysis of copy number variation using formalin-fixed paraffin-embedded (FFPE) samples on BAC microarrays

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Array-based comparative genomic hybridization (aCGH) has become a powerful tool to analyze DNA copy number changes. Especially in combination with archival tissue samples with well-documented follow-up data, it enables the analysis of genomic changes underlying tumour development. Unfortunately, DNA isolated from archival, formalin-fixed, paraffin-embedded (FFPE) material, is often degraded and therefore difficult to label. In theory, labelling techniques that skip the use of enzymes would be the best option. Therefore, we compared the non-enzymatic Universal Linkage System (ULS) and conventional random priming to label DNA isolated from FFPE material. Testing of FFPE samples that had been stored for up to 17 years showed

that ULS-labelling yielded satisfying degrees of labelling (DoL~1.5%), while the ones for random priming were ~0.2%. Reproducibility of the analysis of these samples on BAC arrays was better as well (R<sup>2</sup>=0.96 for ULS vs R<sup>2</sup>=0.88 for random priming).

In addition, aCGH applications involve substantial amounts of genomic DNA, while the amount of DNA available is often limited, requiring whole-genome amplification (WGA). Recently, Qiagen's REPLI-g has been optimized for degraded samples. Therefore, we compared BAC aCGH experiments using 100ng of FFPE DNA, labelled using either direct random priming or a combination of REPLI-g and ULS or REPLI-g and random priming. Correlation with data obtained using conventional microgram amounts of input DNA data was best for the REPLI-g/ULS combination (R<sup>2</sup>=0.84 vs 0.70 for random priming), as was reproducibility.

In conclusion, ULS allows aCGH analysis of archival DNA, while minimizing labelling-induced bias.

#### P08.31

##### Functional SNP candidates identified by genome wide ChIP analyses

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Disease-associated SNPs identified in large association studies are often located in non-coding regions and it is possible that some SNPs affect the interaction between transcription factors and DNA. In HepG2 cells we mapped binding sites for the transcription factors USF1 and USF2 along the genome using ChIP-chip. Using the novel BCRANK algorithm, we detected 1754 binding sites for USF1 with the predicted binding motif. In dbSNP, we found 140 candidate functional SNPs in these motifs. Eight of the SNPs were heterozygous in at least one of four human cell samples. In 5 of 6 cases where the SNP was inside the core predicted USF1 binding motif CACGTGAC, ChIP and sequence analysis of the enriched DNA showed that USF1 was, with statistical significance bound to the allele containing the USF1 consensus and less to the other. We detected significant allelic differences for USF2, POLR2A and H3K27me3. As a negative control, we analyzed 2 cases where the SNP was located just outside USF1 core sequence and saw no differences for any investigated factor. By high resolution genotyping in the HepG2 genome we have a dense map of potential functional SNPs. We have mapped additional factors along the genome using ChIP-sequencing, identified individual binding motifs containing hundreds of SNPs. Furthermore, we found significant enrichment of one allele at heterozygous positions as indication of allele specific DNA-protein interactions. Thus, individual binding sites can be defined at base pair resolution and large numbers of candidate functional SNPs can be predicted in genome wide experiments.

#### P08.32

##### TaqMan® Express: A format for easy mRNA quantification using pre-plated TaqMan Gene Expression Assays

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TaqMan® Express Plates contain up to 96 pre-spotted and dried Taq-Man Gene Expression Assays on an optical 96-well plate. Pre-plated assays simplify, accelerate and help error-proof mRNA quantification experiments. This is especially important when there is more than one operator in a lab, when a study is being done across multiple labs, or when a study is extended over a period of time. The assays in Taqman® Express plates are user-selected from a catalogue of >50,000 Taqman assays targeting human, mouse, rat, Rhesus and dog genes. Here, we show that TaqMan assays which are aliquoted and dried for storage perform comparably to standard wet assays aliquoted immediately before use. Dynamic range for dried assays showed >6 logs of linearity, detection of 2-fold discrimination with 99.7% confidence was demonstrated, and limit of detection was <10 copies. When the wet vs dry performance of a defined set of assays was compared, the difference between the normalized CT values (ddCT) was <0.3. The data also shows strong reproducibility for replicates within and

across plates and across manufacturing lots. For assays with CT<32 the StDev was <0.3 for all of these comparisons. Finally, data showing reproducibility for plates run by different individuals at different sites will be presented.

#### P08.33

##### EUREXpress, a transcriptome atlas of the developing mouse embryo: a valuable resource to study mechanisms of gene expression

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Genome-wide expression analyses have a crucial role in functional genomics. The EU-funded EUREXpress consortium has generated a transcriptome-wide acquisition of expression patterns by means of ISH and established a web-linked, interactive digital transcriptome atlas ([www.eurexpress.org](http://www.eurexpress.org)). To date we have generated over 12000 expression patterns, which have been thoroughly annotated. This interface includes 1420 anatomical structures and correlative trees regarding ontological (embryological) and topological relations allowing advanced queries. In order to perform more global analysis we divided the genes studied into two main categories, based on their annotated expression patterns, namely a) "regional", i.e., genes with clearly detectable patterns which however were not widespread to all the tissues, and b) ubiquitous/undetectable, i.e., genes which either did not give any clearly detectable pattern in any tissue or were detected in all tissues. The analysis of the data has determined that 35-40% of genes at E14.5 present a restricted/regional expression pattern. We sought to establish possible correlations between the genomic properties of regional genes vs. undetectable/ubiquitous genes including gene size, number of annotated alternative transcripts, and degree of evolutionary conservation. Statistical analysis revealed that regional genes have a) an average genomic length considerably higher than undetectable/ubiquitous genes, a) a higher number of alternative splice isoforms and c) are conserved in a higher number of species across evolution as compared to undetectable/ubiquitous genes. In addition, the data has also allowed performing detailed molecular characterization of the CNS and has identified, for example, novel molecular regionalization of the thalamus, diencephalons and the telencephalic pallium.

#### P08.34

##### Functional exploration of the fibrinogen gene landscape to identify new regulatory mechanisms

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The transcription of the three fibrinogen genes is coordinately regulated and previous studies have described cis-acting elements situated in each of the 5'-promoter regions. Recent advances in vertebrate genome sequencing and annotation allowed us to search for more distant cis-/trans-regulatory elements involved in the regulation of the fibrinogen gene cluster and investigate the molecular mechanisms by which these elements participate in transcriptional regulation. Multi-species genome comparisons revealed remarkable conservation of the fibrinogen gene cluster structure as well as a conserved adjacent syntenic gene desert region (130kb). We therefore speculate that regulatory element of the fibrinogen gene cluster have also been conserved throughout the vertebrates' evolution and reside in the syntenic regions including the cluster. Analyses of the conserved block of synteny bearing the fibrinogen cluster (200kb), using the VISTA browser, revealed 33 conserved non-coding sequences (CNC). We tested the regulatory potential of 17 of the CNCs in a reporter assay, by inserting each CNC upstream of a Luciferase gene. Regulatory activity was measured in hepatoma cell lines (Huh7, HepG2) and in HEK-293T cells. We identified 3 CNCs with hepatocyte-specific enhancer activity, one residing between FGB and FGA and two situated in the adjacent syntenic non-coding region. We are also performing chromosome conformation capture (3C) experiments to identify cis or trans DNA-DNA interactions between the proximal fibrinogen gene promoters and currently unknown sequences.

Future work will focus on the functional characterization of these newly identified cis-regulatory elements.

**P08.35****Complete sequencing of the CFTR gene using the new generation GS-FLX sequencing technology****H. Cuppens, L. Vliegen, J. Cassiman;***Center for Human Genetics, Leuven, Belgium.*

In most genes involved in genetic diseases, a broad spectrum of mutations is found. Even for diseases such as cystic fibrosis, genetic testing can be very challenging. Indeed, routine CFTR genetic tests only screen for the most common mutations (88-92% sensitivity in most European countries).

New generation sequencing technology, such as picotiter pyrosequencing on a GS-FLX system, has been recently introduced. However, this technology was initially developed for whole genome sequencing purposes.

We adopted this technology for complete sequence analysis of the CFTR coding region, and its exon/intron junctions.

To this aim we have developed a robust multiplex amplification assay in which biotinylated amplicon-specific primers are locally restricted through streptavidin/biotin crosslinking. Indeed, 30 amplicons should be analyzed for the CFTR gene, which can be only economically feasible if amplified in one, or a limited number, PCR multiplex reaction(s). For a 50x coverage, only half a million nucleotides are needed for CFTR sequence analysis, i.e. 0.5% of the full capacity of the GS-FLX system. Therefore, 100-200 samples should be pooled in order to use the full capacity of the GS-FLX system. We therefore also developed an universal sample tagging approach allowing the pooling of more than 100 samples with one set of 260 primers (60 amplicon-specific and 200 tagging primers). This compares to 6000 primers if amplicon-specific PCR primers are tagged as such.

This technique is readily transferable to any gene, allowing sequencing of more than 100 samples for the same gene, or even different genes, in an economically feasible way.

**P08.36****Nuclear localization of SM22 alpha during heart development****E. Bregant<sup>1</sup>, R. Lonigro<sup>1</sup>, N. Passon<sup>1</sup>, A. Scaloni<sup>2</sup>, G. Renzone<sup>3</sup>, M. Pandolfi<sup>4</sup>, C. Di Loreto<sup>4</sup>, G. Damante<sup>5</sup>;**

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The molecular mechanisms that control heart development have been the subject of intense investigation. The transition of embryonic to adult cardiomyocytes is associated to changes in the expression patterns of different proteins.

Aim of this study is the identification of nuclear proteins whose expression is modified during cardiac differentiation. A proteomic approach, based on two-dimensional electrophoresis was utilized. The experimental model is the H9C2, a myoblast cell line derived from embryonic rat ventricle. These cells proliferate in medium with 10% serum, instead low serum and stimulation with retinoic acid induce differentiation versus cardiomyocytes.

We have analyzed the nuclear extracts of H9C2 that are grown in proliferation medium and in differentiation medium by proteomic approach.

Seven different proteins have been identified as differentially expressed after MALDI-TOFF, LC-ESI-MS/MS mass spectrometry. An interesting protein is SM22 alpha (transgelin), a 22-KD cytoskeletal protein that is a marker of smooth muscle cells.

The level of the SM22 alpha is reduced at 20<sup>th</sup> day of differentiation condition. These data are confirmed by Western-Blot analysis and quantitative RT-PCR.

By immunochemistry, we confirmed the nuclear localization of the protein in H9C2 cell line. Furthermore, in histological section of human embryonic heart we show that SM22 alpha is located at nuclear level in heart vessels and in myocytes of the cardiac outflow.

Thus, our data indicate that SM22 alpha can be localized in the nucleus and suggest that this localization is regulated during development

**P08.37****Methylation-sensitive High Resolution Melting Analysis as a diagnostic tool for Beckwith-Wiedemann and Silver-Russell syndromes.****M. Alders, J. Bliek, K. vd Lip, R. vd Boogaard, M. Mannens;***Academic Medical Center, Amsterdam, The Netherlands.*

The Beckwith-Wiedemann syndrome (BWS) and Silver-Russell syndrome (SRS) are caused by disturbed imprinting at 11p15. This region harbors two independently regulated clusters of imprinted genes. The first cluster is under control of the IC1 upstream of the H19 promoter, which is methylated only at the paternal allele. The second cluster is controlled by IC2 upstream of the KCNQ1OT1 promoter and is methylation on the maternal allele only.

BWS is an overgrowth syndrome. The majority of the patients display hypermethylation of IC1, hypomethylation of IC2 or both. SRS is a growth retardation syndrome and in a subset of patients a hypomethylation of IC1 is found, opposite to the aberration found in BWS patients.

Molecular confirmation of BWS and SRS is done by methylation analysis of IC1 and IC2. Since the methylation defects in BWS and SRS are mosaic the test must be quantitative.

We set out to validate High Resolution Melting Analysis (HRMA) for methylation analysis in BWS/SRS diagnostics. Advantages of this method are that it is fast, cost effective and requires no post PCR handling. We tested a group of 17 BWS/SRS patients with different levels of hyper- and hypomethylation at IC1 and/or IC2 and 45 normal controls. All patients showed a melting profile different from the normal controls and the degree of deviation was consistent with the degree of hypo- or hypermethylation as determined by southern blotting. In conclusion, HRMA analysis proofs to be a fast, reliable and sensitive diagnostic tool for BWS and SRS.

**P08.38****Calibration improves methylation-sensitive high resolution melting results****C. N. Gundry<sup>1</sup>, M. Wall<sup>1</sup>, J. McKinney<sup>1</sup>, J. D. Phillips<sup>2</sup>, M. K. Yu<sup>3</sup>, D. H. F. Teng<sup>1</sup>;**

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High resolution melting has been shown to be a sensitive method for methylation detection of CpG sites. In high resolution melting, multiple CpG sites within the same PCR fragment can be detected homogeneously. Unlike the common real-time methylation-specific PCR technique (MethylLight Taqman probe detection) hi-res melting provides more information with fewer PCRs. As 5-methylcytosines are resistant to conversion to uracils, there can be substantial sample-to-sample melting differences depending on the number of CpG dinucleotide sites and the sequence context within the fragment. However, depending upon fragment and the exact number of 5-methylcytosines actually converted during the bisulfite treatment, there can be both variation in replicate conversions of the same sample and very subtle melting differences between samples. Both of these problems are resolved with calibrated melting. Calibration has especially been helpful to increase accuracy of genotyping when melting differences are subtle. This techniques improves hi-res melting reproducibility and genotyping calls via synthetic oligonucleotide probes. We used melt calibration in conjunction with amplicon methylation analysis to improve our detection in a highly methylated region. We obtained excellent resolution of amplicon fragments within a hypermethylated region of the miRNA-195 genomic sequence. Sequence verification showed that our homogeneous technique is comparable to this gold standard in accuracy.

**P08.39****Interlaboratory validation of High Resolution Melting (HRM) for BRCA1 and BRCA2 on the LightCycler® 480****T. Janssens<sup>1</sup>, N. van der Stoep<sup>2</sup>, R. Buser<sup>2</sup>, G. Michiels<sup>1</sup>, A. Corveleyn<sup>1</sup>, E. Dequeker<sup>1</sup>, P. Mailler<sup>3</sup>, E. Bakker<sup>2</sup>, G. Matthijs<sup>1</sup>;**

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High Resolution Melting (HRM) was selected as a technology for which a thorough validation would be very timely. In a collaborative EuroGenet test study, we extensively tested it on the LightCycler® 480.

HRM is a fast, simple and cost-effective high-throughput scanning

method to detect sequence variations. PCR is performed in the presence of a saturating dsDNA binding dye. Single-base variations in the amplicon result in altered melting behaviour after heteroduplex formation, visualised by HRM plots. The LightCycler® 480 performs both PCR and HRM in one run.

BRCA1 and BRCA2 were chosen as target genes, because their size and mutation spectrum represent a challenge in molecular diagnostics. Current methods, like dHPLC and DGGE, despite their good performance, are limited by throughput or labour-intensity.

HRM is potentially useful for solving these problems. However, it needs to be shown that the sensitivity and the user-friendliness are at least as good as the current state of the art. We set up an extensive validation in 3 laboratories.

The first objective was to design a complete primer set for BRCA1 and BRCA2. Indeed, the performance of HRM is largely depending on PCR quality. Critical criteria were specific banding patterns after gel electrophoresis, sigmoid curves on real-time PCR plots and less than 3 melting domains per amplicon.

About 300 known variations are being tested in a blinded way. This will allow us to determine whether HRM reaches a sensitivity close to 99%, which would make it a suitable new method for diagnostic use.

#### P08.40

##### Interlaboratory validation study of High Resolution Melting Curve Analysis for mutation scanning of BRCA1 using the LightScanner (IT)

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The current set up for mutation scanning of BRCA1 occurs through sequence analysis, DGGE, PTT and DHPLC. All these techniques are time consuming and expensive. Therefore we have evaluated the High-Resolution Melting Curve Analysis (HR-MCA) as a high-throughput mutation-scanning tool for the BRCA1 gene using LightScanner from Idaho Technology (IT) and the LightScanner-MasterMix. This study was implemented in the EuroGentest evaluation program for new techniques in genome diagnostics. Investigations were carried out using a panel of >189 variants and 327 wt controls. We optimized and evaluated 58 primer-sets that cover BRCA1. All heterozygous variants could be detected using the Call-IT-1.5 software resulting in a 100% mutation-detection sensitivity. These variants also include small DNA deletions and insertions. Out of 327 wts we observed 3,7% false positive curves (FP) resulting in a specificity of 96%. Re-evaluation of ten BRCA1-amplicons in the two other diagnostic laboratories gave rise to identical results. Moreover using unlabeled probes we were able to identify many frequent occurring polymorphisms, omitting unnecessary sequence analysis. Finally we selected a set of 41 best-performing primers that encompass the entire BRCA1 gene using stringent criteria and performed 2 blind tests on 27 patient DNA samples that were sequenced in parallel. Optimal HR-MC analyses setting were fixed based on the results obtained with our large set of known control samples. In both series we detected all heterozygous variants and observed a FP-score of 1,8%. In conclusion, HR-MCA with the LightScanner appears to be a rapid and sensitive method for mutation scanning.

#### P08.41

##### Isolation and transcriptional profiling of embryonic human neural crest cells

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Developmental and stem cell biology ask the common question of how functionally distinct cell types arise from one self-renewing founder population. Human neural crest cells (hNCC) form in the embryo at the end of the first month of pregnancy, although the precise dates have remained elusive. Most NCC differentiate completely into melanocytes, all components of the peripheral nervous system, certain endocrine cells and connective and structural tissues in the head, although par-

tially competent NCC progenitors continue to reside in many tissues in animal models. Due to their inaccessibility and despite their developmental importance, the original properties and restriction of hNCC potential over time have never been examined. Here, we demonstrate when and how to isolate self-renewing hNCC and report the resultant study of their transcriptome by serial analysis of gene expression (SAGE). In addition to highlighting candidate disease genes, we have identified novel markers that distinguish hNCC from other human cell types and distinguished between evolutionarily conserved and species-specific NCC properties. Genome-wide analysis, using multiple statistical criteria, demonstrates that the global molecular signature of early migratory hNCC is remarkably similar to that of pluripotent human embryonic stem cells. We show that most cell types of the human pharyngula express a number of genes *in situ* that, individually, do not autonomously confer pluripotency but taken within the context of the transcriptome, may be permissive for the unique plasticity of hNCC over time. Our findings pave the way for further studies of hNCC therapeutic potential in neurocrustopathies such as peripheral demyelinating disorders.

#### P08.42

##### Human Leukocyte Antigen (HLA) Typing and Sequence-Based Typing (SBT) for Blood Stem Cell Transplants

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Human leukocyte antigens (HLA) are protein markers that are found on most of the body's cells. The immune system uses HLA to recognize which cells belong in your body and which do not. These protein markers help identify a person's tissue type. The HLA proteins are important in matching patients and donors for a bone marrow, peripheral blood cell (PBSC) or cord blood transplant. When a transplant center looks at the match level, it is looking at how alike the HLA tissue types of the patient and the donor are to each other.

We will demonstrate that directed sequencing of over 40 PCR-amplicons (including forward/reverse) in the HLA-Class I region provides a complete test result for each patient. Even one amino acid variation in each HLA gene between the donor and recipient increases transplant mortality risk by 10%. Using an exact sequencing match will greatly improve the patient's chances of transplant success.

Direct sequencing for HLA typing gives the accurate and ultimate answer, without a timely screening step prior to sequencing. Using state-of-art automated capillary electrophoresis instrumentation and bioinformatics tools to deliver these results saves lives by reducing time before a donor is located, increasing successful transplant outcome and lowering subsequent healthcare cost.

#### P08.43

##### Conserved and non-conserved transcription regulatory elements associated with SINEs in mammalian promoters

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About half of promoters of protein-coding genes in the mouse and human genomes contain sequences of interspersed repeats which can effect gene expression. Recent estimations suggest that some of these sequences are evolutionary conserved and may represent >5% of all constrained nonexonic elements which are under strong purifying selection in mammalian genomes. However, major fraction of important regulatory elements is not constrained in evolution. These elements can be recruited from a large pool of lineage-specific dispersed repeats. For example, human Alu and mouse B1 repeats contain short conserved segments with potential binding site for transcription factor PitX2 (binding motif YTGGGATTANW) which is important for establishment of left-right asymmetry in development of multiple organs. Alu-associated binding sites for PitX2 regulate expression of human PLOD1 and ANF genes but most of downstream target genes for PitX2 is not established. Our results indicate that >1000 of mouse or human genes contain Alu/B1 associated PitX2 binding sites and these genes are expressed in almost all tissues. >100 of these promoters also have potential binding site for transcription factor Nkx2.5 (motif TYAAGTG) which frequently cooperates with PitX2 in gene regulation. Among PitX2 and Nkx2.5 targets in the mouse genome we found promoters of SMARCD3, PRKCZ and NIPA1 genes expressed at high level in

brain. However, in human genome other brain-expressed genes having SINE-associated PitX2/Nkx2.5 sites are identified (e.g. KIF17). It is likely that SINEs contributed to regulatory evolution of brain-expressed genes through their associated binding sites for PitX2 in a lineage specific manner.

#### P08.44

#### KingFisher® Flex - Versatile automated nucleic acid, protein and cell purification

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*Thermo Fisher Scientific, Vantaa, Finland.*

Sample preparation is often a limiting step for genomics and proteomics studies. Rapid and efficient isolation of nucleic acids, proteins and cells from complex biological matrixes is needed to get high quality starting material for various experiments. KingFisher, the magnetic bead based, automated purification system from Thermo Fisher Scientific, provides a quick and easy solution to achieve high quality and reproducible results in purification of nucleic acids, proteins and cells with minimal hands-on time. The technology is based on magnetic rods which move particles through the various purification phases - binding, mixing, washing and elution. The KingFisher is an open and flexible system, letting the user to choose any available magnetic particle based purification kit suitable for the application.

Thermo Scientific KingFisher family consists of three instruments with different throughput and working volume capacities. The latest member of the family is KingFisher Flex, which is truly flexible solution for different types of sample processing needs. There are four magnetic heads available for the instrument depending of the needed processing volumes. With the 24-rod configuration the processing volume can be raised up to 5 ml and with the 96-rod configuration it is possible to achieve the highest throughput in working volumes 20-1000 µl. The instrument is compatible with robotic platforms providing a high throughput automated solution for all biotech and pharmaceutical laboratories. The data from different applications shows that KingFisher Flex provides a rapid method for purification of high quality and quantity biological molecules.

#### P08.45

#### Diagnosis optimization by using MLPA in the investigation protocol in mentally retarded children - Romanian experience

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Mental retardation (MR) is a heterogeneous entity, genetic causes being involved mainly in moderate/severe forms. Subtelomeric rearrangements (SR) play an important role in idiopathic MR determinism. Recently introduced MLPA (Multiplex Ligation Probe Amplification) technique seems to provide good results in SR's identification. We have used MLPA to identify SR in children with idiopathic MR, the protocol consisting of the following sequence: clinical selection based on de Vries score; karyotype; antiFMRP test for Fragile X syndrome screening; PCR for Fragile X syndrome diagnosis; MLPA testing using 2 independent kits in order to separate polymorphisms. Parents were tested if an abnormality was detected in their child (by karyotype/MLPA). The study group was formed of 223 children that had a de Vries score of 3/more. All patient data were recorded in a database specially designed. In 28 of them (12.5%) the karyotype revealed different abnormalities. 17 cases (7.6%) presented speech delay/ autism, but anti-FMRP test and PCR were normal. Out of the 195 MLPA tests done: 168 cases (86.1% were normal, 13 (6.7%) abnormal and 14 (7.1%) had polymorphisms. The most frequently involved SR were 1p del and 7q del. Clinical features of the cases identified with different SR will be illustrated with photos and discussed.

In conclusion, we appreciate that the diagnostic score is useful in case selection for further testing, MLPA proves to be efficient in diagnosing SR and the frequency of SR as a possible cause of idiopathic MR is similar to other studies in the literature.

#### P08.46

#### Confocal morphometry of the transfected human mesenchymal stem cells

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Transfected human stem cells (SC) demonstrate great potential for clinical applications. They are extensively investigated to elucidate their efficiency and safety. We focused on studying the reaction of SC on the introducing the genetic construction. Morphometry of nuclei of the transfected mesenchymal stem cells (MSC) was performed.

**Materials and methods:** The culture of MSC at the "late" passage (12) was used. Lipid-mediated transfection was performed with a non-viral vector based on ps415 containing VEGF121 using UF-56 as the lipid media. After 90 minutes media was changed. Cells were washed and cultivated for two days, then seeded on cover-glasses and cultivated 24 hours more. Cells were fixated with paraformaldehyde. Nuclei were contrasted with DAPI. Scanning was performed using confocal microscope Axioimager A1.

**Results:** average nucleus dimensions of the transfected human mesenchymal stem cells: 14,6x8,7x8,3mkm and of control MSC - 14,8x8,9x8,3mkm.

**Conclusions:** transfected MSC have the same nucleus dimensions as the control MSC. Additional studies should be performed to check chromatin structure and the form of the nuclei which appeared slightly different in our study. Objective formal criteria should be formulated to perform such analysis. The integrity of the nucleus may prove the stability of MSC after transfection forming the basis for further application of the transfected MSC in clinical trials.

#### P08.47

#### RMetaWeb: Meta-analysis online tool

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Meta-analysis is a statistical procedure that integrates the results of several independent studies. The number of papers published on meta-analyses in biomedical domain has increased sharply in the past 15 years. The merits and perils of the somewhat mysterious procedure of meta-analysis, however, continue to be debated in the medical community. Given the increasing pace of published meta-analysis studies, their methodological quality, however, still leaves much to be desired. We have developed RMetaWeb, an online system for comprehensive, fast, and reliable research synthesis analysis. The web interface is based on a Perl CGI script that communicates with R using the CGIwithR library. An interactive viewer provides straightforward navigation through various procedures. Output is exported to an HTML and PDF, which offers convenient views on the results in both tabular and graphical formats. RMetaWeb is intended to serve to the scientific community and it is hoped that it will become a useful tool on conducting reliable meta-analysis in biomedical research. The RMetaWeb is freely available from <http://genepark.mf.uni-lj.si>.

#### P08.48

#### Optimizing bisulfite DNA conversion method for methylated CpG island discovery and screening

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DNA methylation at the 5' position of cytosine in promoter CpG islands plays a critical role in regulating gene expression. Typically methylation is inversely correlated with the transcription status of the gene. Bisulfite DNA conversion is one of the most used techniques for methylation studies because of its relative simplicity, whereas other methods are frequently cumbersome and require significant optimization. The bisulfite conversion method allows precise analysis of methylation in a target region by converting all nonmethylated cytosines into uracils, while methylated cytosines remain unchanged. The workflow described here provides an effective solution for methylation analysis with straightforward protocols. We recognize that bisulfite conversion rate and primer design are the most critical steps in this approach. In this presentation, we demonstrate that a promoter region's methylation status can be identified with confidence using an optimized approach, which requires small amounts of genomic DNA and generates high quality data. This workflow, therefore, is very useful for the analysis of

clinical samples, where the amount of material is limited and analysis time is an important factor.

#### P08.49

#### Combined analysis of altered promoter methylation and gene expression changes in non-small cell lung cancer

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Combined analysis of promoter methylation and gene expression helps to reveal causative links between the epigenotype and phenotype of malignant tumours. Methylation changes in cancer are frequent and relatively stable, therefore they're also attractive biomarkers for tumour profiling, as well as potentially for monitoring the efficiency of anticancer therapy.

We have performed an analysis of 48 genes in 60 surgically treated lung adeno- and squamous cell carcinoma patients, using *in situ* synthesized oligonucleotide microarrays. The samples were analysed for promoter methylation, as well as the corresponding gene expression changes. The analysis included known and putative tumour suppressor genes, genes controlling cell growth and differentiation, antiangiogenic factors and genes participating in cell to cell and cell to extracellular matrix connections. A panel of tumour-free lung tissue was used as the negative control. The dual level data was analysed with M-CHIPS platform and showed clear differences between cancer and cancer-free tissue.

A similar study with more complex setup is underway in a different clinical center.

#### P08.50

#### The new MLPA based approach for HLA-DQA1, -DQB1 typing

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The genetic complexity of the human major histocompatibility complex (MHC) has required the using of various molecular typing methods to identify specific alleles. Sequence specific primers (SSP), sequence specific oligonucleotide probes (SSOP) and sequence-based typing (SBT) are a few of the methods that have been utilized in the HLA community at present. We have designed Multiplex ligation-dependent probe amplification (MLPA) method based system for HLA-DQA1 and -DQB1 loci typing. Such MLPA advantages by comparison against others molecular method as cheapness and simplicity of the technique and a possibility to determine chromosome haplotype are governing for HLA typing method. Our MLPA based system reveals 8 DQA1 alleles in one tube and 19 DQB1 alleles in one tube. A working procedure is consisting of three steps; the site-specific oligonucleotide probes ligation, multiplex amplification with common primers pair and PCR fragments assessment. For comparison, our earlier SSP based system reveals the same alleles; the working procedure includes 2-4 PCR/Multiplex PCR during HLA-DQA1 typing and 5-8 PCR/Multiplex PCR during HLA-DQB1 typing per person. In our option MLPA method is excellent additional molecular HLA typing method.

#### P08.51

#### NASBA product labeling for following microarray experiments

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DNA microarrays that enable quick and simultaneous investigation of many different biological targets are steadily becoming everyday tools in genetics and molecular diagnostics. Environmental and medical samples can easily be identified and investigated by specific probe microarrays that are complementary to the suitable marker sequences in analyzed solution. Biological markers of interest are usually amplified and labeled prior to the microarray hybridization experiment. RNA targets can efficiently and exclusively be amplified using nucleic acid sequence based amplification (NASBA), even in the presence of genomic DNA. NASBA is an isothermal method that has previously

been applied for the detection of different pathogens and mRNAs. Several labeling protocols for many amplification methods have been described in literature previously, but none of them are for NASBA amplification products. Commercial NASBA amplification method was modified to meet the needs of microarray experiment. Extra aminoallyl-UTPs were added to the NASBA solution in order to add fluorescent marker Cy3 to the aminoallyl modified RNA products after the reaction. Two different commercial aaUTP reagents (aaUTP-Na and aaUTP-Li salts) were investigated over wide concentration range (0,125mM to 8mM). Strongest microarray signal intensities were achieved with 2mM final concentration of aaUTP-Li salt used in NASBA reaction. Modified NASBA method enabled accurately detect pathogens from solution containing total RNA from as low as 0,1-1 CFU.

#### P08.52

#### Identification of mir-21 (MIRN21) targets in breast cancer cells by gene expression profiling

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Increasing numbers of microRNAs have been shown to target cancer related genes. Our aim was to identify mir-21 targets which are regulated through mRNA cleavage, deadenylation, or other processes that can lead to mRNA up- or down-regulation. Our experimental approach involved silencing of endogenous mir-21 expression by 2'-Ome-inhibitors (anti-mir-21) in MCF7 cells. Total RNAs from anti-mir-21 transfected, control oligo transfected and untreated cells were further processed and hybridized to Affymetrix Human Genome U133 Plus 2.0 arrays. Statistical and clustering analyses were conducted using Bioconductor to identify the effects of mir-21 silencing on the transcriptome. Using p<0.05 and fold change>1.5 as cut-off values, 134 transcripts were differentially expressed only in anti-21 treated samples. The transcriptome responsive to mir-21 silencing was analysed further by GO (gene ontology) based clustering of functionally related genes and biochemical pathway analysis (GO, KEGG) to gain better understanding of mir-21 function in cancer pathways. Over 25 % of the mir-21 sensitive genes were involved in regulation of transcription, 9.62 % in apoptosis and regulation of programmed cell death, while the rest were involved in a range of other biological processes. Amongst these, some genes were linked to apoptosis by insulin signalling, cell cycle regulation by TGF-BETA and MAPK signalling pathways suggesting a potential involvement of mir-21 in regulatory control of these cancer-related pathways. Further studies are underway for verification candidate targets identified. These studies will facilitate the identification of new cancer biomarkers regulated by microRNAs that could be used for better diagnostic and therapeutics for breast cancer.

#### P08.53

#### A Pipeline for Designing Custom TaqMan® Assays for Small RNA Genes

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MicroRNAs (miRNAs) are a new class of non-coding RNAs that mediate post-transcriptional gene silencing. A growing number of novel miRNAs and other small RNA genes are being discovered and there is a significant need for custom assays to determine the level of their expression. An automated bioinformatics pipeline has been developed to design TaqMan® MicroRNA Assays to allow quantitation of miRNA expression by real-time PCR. To date, over 2,000 assays have been designed using the pipeline for miRNAs listed in miRBase (release 10.0). The pipeline has been wet-lab validated for mammalian miRNAs showing assay performance success of greater than 90% based on assay linearity and no template control (NTC) background signal. As research interest in small non-coding RNAs is rapidly expanding beyond miRNAs, the capability of the pipeline to design assays for other small RNAs including small interfering RNAs (siRNAs), short hairpin RNAs (shRNAs), and PIWI-interacting RNAs (piRNAs) is extremely valuable. Assays for over 100 endogenous siRNAs have been designed using the same design pipeline as for miRNAs. Preliminary data shows similar performance as miRNA assays with minimal background in the presence of tissues and cell lines for these siRNA quantitation assays. In conclusion, we have developed a robust pipeline to successfully design TaqMan miRNA assays. Recent data demonstrates the ability

of the pipeline to design TaqMan assays for quantitation of siRNAs as well as the potential for designing assays for other small RNA genes.

#### P08.54

#### Human mitochondrial DNA data integration with the semantic web

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Human mitochondrial DNA (mtDNA) is a 16.5 kb circular genome in which numerous polymorphisms have been detected in thousands of sequenced genomes from various populations and patient groups worldwide. Evolutionary data and the lack of introns in mtDNA suggest that many of these polymorphisms have functional significance, and there are many reports suggesting that some polymorphisms or continent-specific mtDNA lineages (haplogroups) are associated with an increased risk of certain complex diseases or traits. Association data, haplogroup definitions, sequences, polymorphisms and structural and functional features of mtDNA-encoded genes form an immense body of knowledge which is currently scattered around different databases and resources. Current mtDNA databases have been designed principally to be viewed by human researchers and do not provide uniform access to all data and are therefore not optimal for data mining or other purposes where hypotheses are tested against huge amounts of heterogeneous data.

The semantic web is an emerging technology which allows integration of almost any kind of data in a framework which also makes it possible for computers to perform inference tasks in an unified and standardized manner. Mitochondrial Information Integration Initiative (MitoI3) aims at developing an open resource that will collect data on available human mtDNA sequences, polymorphisms, haplogroup definitions and other aspects of mitochondrial genomics. The data are stored as RDF (Resource Description Framework) statements in a RDF triple store which can be accessed with semantic web browsers and which also provides a SPARQL interface for performing complex queries over the data.

#### P08.55

#### The effects of the *MTHFR* gene variations for drug response in rheumatoid arthritis and psoriatic arthritis patients treated with methotrexate

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Methotrexate (MTX) is a folic acid antagonist widely used to treat immunosuppressive disorders such as rheumatoid arthritis (RA). The enzyme 5,10-methylenetetrahydrofolate reductase (*MTHFR*) is involved in maintaining folate and homocysteine homeostasis and common genetic variations, c.677C>T and c.1298A>C, in the *MTHFR* gene are associated with decreased enzyme activity and altered folate levels, which may predispose to increased susceptibility to the anti-folate effects of MTX therapy. Here, we studied the effects of these variations for MTX response in a total of 298 Finnish patients who had either rheumatoid (218) or psoriatic arthritis (80). Together with genotyping we also analyzed folate, homocysteine, ALAT, and B12 vitamin levels as well as clinical patient data concerning toxicity and efficiency of the MTX treatments. Finally, statistical analyses were done to evaluate possible associations between the *MTHFR* gene variations and MTX toxicity and efficiency. Our results show that the patients with two normal alleles (677TT/1298AA) are less likely to experience side effects during MTX therapy although, no statistically significant association between variations and methotrexate toxicity was found. Remarkably, *MTHFR* 677TT was recognised to be a risk genotype for elevated ALAT as well as a serious risk factor for low folate and accordingly elevated homocysteine levels both during and after MTX treatment. In addition, the state of the disease stayed remarkably more often active in patients having 677TT genotype and low folate levels suggesting that *MTHFR* c.677C>T is critical genotype and should be taken into account in patient treatment strategy.

#### P08.56

#### Denaturing electrophoresis on Lab-on-Chip Agilent BioAnalyzer platform: A novel approach for rapid screening of DNA mutations

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Recent expansion in genome research brought a number of new analytical technologies for detection of DNA mutations and polymorphisms. Although many exciting approaches are presented in scientific environment, only a relatively small fraction subsequently finds its way to practical application in routine diagnostic testing. Among the most widely used methods are either enzymatic methods such as RFLP, OLA, TaqMAN, etc. or methods based on separation due to secondary DNA structure effects such as DGGE, TGGE, SSCP, dHPLC and others.

Over the past several years, many of the electrophoresis-based techniques have been adapted to capillary electrophoresis DNA sequencers utilizing separation under partial denaturing conditions at either constant-temperature or in temperature gradients settings.

In the current work we introduce a DNA melting separation on an electrophoresis chip platform. We adapt Agilent 2100 Bioanalyzer for detection of common point mutations in Factor V Leiden and Factor II Prothrombin genes. The technique employs denaturing temperature gradients on the chip to reliably separate homo- and hetero-duplex forms.

This work was supported by the Czech Ministry of Industry grant FI-IM3/215.

#### P08.57

#### Web-based collection of gene-specific sequence variants and their phenotypic consequences

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Soon it will be possible to sequence a human genome for a reasonable price, making medical and clinical application of human genome sequencing a realistic option. However, as highlighted by the Human Variome Project meeting in Melbourne (2006), when we want to understand the consequences of all the variation we will see, we need to improve significantly in reporting and cataloguing the variants and their consequences we identify at the moment. To facilitate the collection of sequence variants and their phenotypic consequences we have developed the Leiden Open-source Variation Database (LOVD) software ([www.LOVD.nl](http://www.LOVD.nl)). LOVD provides a free, open-source, platform-independent and fully web-based tool to build, curate and share Locus-Specific mutation DataBases (LSDBs). In addition, to facilitate error-free reporting of variants we have developed the Mutalyzer nomenclature checker ([www.LOVD.nl/mutalyzer](http://www.LOVD.nl/mutalyzer)) and coupled it to LOVD. Mutalyzer names sequence variants following the HGVS mutation nomenclature recommendations, using a GenBank accession number, a HGNC gene symbol and the variant as input. Currently, our Leiden server hosts over 70 gene variation databases, with data collected for more than 20,000 patients contributed by 150 submitters world-wide, the largest series covering gene variants in relation to neuromuscular disorders.

#### P08.58

#### Next Generation Sequencing

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The next generation sequencing technologies, embodied by the Roche Diagnostics/454, Illumina/Solexa and Applied Biosystems sequencing machines, provide the opportunity to generate huge amounts of sequence information for de novo and resequencing of genomes. GATC uses all three leading high throughput sequencing machines in house: the GS FLX from Roche Diagnostics, the Illumina Genome Analyzer and the SOLiD system of Applied Biosystems, providing the flexibility to tailor sequencing projects to meet customer requirements. The bioinformatic analysis of the huge amount of data is still a challenge, especially for the human genome (3 GB). In addition, many questions regarding cancer research or disease related topics, can be addressed by sequencing just the area of interest of the human genome. Often these areas are too big to be amplified out of the whole genome. GATC

will present data from enrichment studies of the human genome of sequence areas related to different diseases. The paired-end method for the technologies provides additional information about large scale variations in the genome allowing an accuracy approaching classical Sanger sequencing. Comparison of the next-gen sequencing data to a reference genome provides a mapping that takes advantage of the high coverage to identify SNPs and other structural differences and variations. The results can be imported into other assembly programs (e.g. SeqMan™ of the Lasergene™ suite from DNASTAR Inc., USA).

#### P08.59

#### A novel approach for miRNA profiling and discovery using massively parallel ligation-based dibase sequencing technology

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Next-generation sequencing (NGS) platforms produce 10-100's of millions of short reads (25-50bp) in a single run which makes them particularly suited for tag counting application including gene expression profiling. With Applied Biosystem's new platform, sequencing is carried out via dibase sequential rounds of ligation with high fidelity and high read quality. Using a newly developed library protocol which requires low sample input and results in sequence ready samples in less than a day, we explored the expression profiles of small non-coding RNAs in two normal tissues, using the SOLiD™ system. The frequency and distribution of miRNAs, isomiRNAs and miRNA\* were evaluated and the fold changes generated from these tissues were compared to those of 380 TaqMan® miRNA assays. Significant correlation levels were observed confirming the applicability of this approach for small RNAs expression profiling. Moreover, more than 3000 potentially novel miRNAs or non-coding RNAs were discovered. These potential novel small RNAs are currently being further validated.

This new library approach coupled with the SOLiD™ System provides a high throughput method for digital gene expression that enables the discovery of novel small ncRNAs and miRNAs as well as profiling their expression levels, without the probe bias of microarrays. Because of the SOLiD™ System's throughput which is greater than 100M reads per slide, it is particularly suited for the analysis of gene expression being able to deliver the dynamic range required to detect genes expressed at very low levels and to accurately measure fold changes at the same low expression levels.

#### P08.60

#### Cloning and construction of mouse PEP cDNA chimera with EGFP under regulation of CMV promoter

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Peroxisomes are tiny organelles in almost all eukaryotic cells. They exhibit various functions including β-oxidation of very long chain fatty acids and metabolite peroxidation which is essential for the cell function and differentiation. We have cloned (Peroxisomal Protein) PEP cDNA in a mammalian expression vector in a chimeric cDNA type, encompassing PEP with EGFP cDNA. Amino acid alignment analysis revealed two hydrophobic domains. The First Comprises twenty amino acid residues between 12-31 residues and the second one, is located at 152-169 residues. There is a tripeptide (SKI) at carboxy terminus responsible for sorting of this protein to the matrix of peroxisome. There is a fibronectin type III domain between residues 31-114 in pep. In order to see the importance of above sorting signal, we performed a site-directed mutagenesis to delete SKI tripeptide. Amplified Pep cDNAs either containing SKI or deleted ones were constructed downstream of EGFP cDNA under regulation of CMV promoter in pEGFP-C1 vector and were send for sequence. Transfection of plasmids containing chimera of EGFP-PEP cDNAs in to the CHO-K1 showed several punctate structures presumably peroxisomes while, SKI deletion showed a cytosolic pattern like EGFP-C1. Taken together, these data strongly suggest that SKI, which is located at the C-terminus of protein is required for sorting of this protein.

#### P08.61

#### The Mouse Genome Informatics (MGI) resource: translating mouse phenogenomics into models of human disease

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The mouse is universally considered a premier model system for studying human development and disease. The MGI resource (<http://www.informatics.jax.org>) provides free access to integrated data on the genetics, genomics and biology of the laboratory mouse, facilitating navigation through sequence, polymorphism, spatiotemporal expression, mapping, biochemical function and process, sub-cellular topology, mammalian homology, phenotype and disease model data. MGI curates aberrant mouse phenotypes in the context of mutations (spontaneous, induced or genetically engineered), strain variations, QTLs, and complex traits that serve as credible models of human genetic disorders, incorporating phenotype-related images as available. Robust querying parameters include standardized terms from the Mammalian Phenotype Ontology, a hierarchically-structured vocabulary that supports morphophysiological annotations to background-specified allelic genotypes at varying degrees of granularity. Parallel use of key bio-ontologies, including the Anatomical Dictionary, GO, and OMIM, fosters innovative approaches to peruse expression profiles, map functional features of gene products to complex pathophysiological states, and establish associations between observed mouse phenotypes and orthologous human gene mutations or distinct nosological entities for which defined mouse genotypes phenomimic the human condition. MGI advances translational research through an integrated data platform which optimizes retrieval and semantic interpretation of multi-parametric, genome-scale datasets, and permits disease model mining from a genotype, phenotype or computational perspective. Recent enhancements include a redesigned homepage and site-wide navigation paradigm, and an image-rich "Phenotype, Alleles & Disease Models" portal, as one of several mini-homepages, each encapsulating a different MGI biomain along with content-specific access instructions, statistics, FAQs, and news announcements. Supported by NIH grants HG000330, HG02273, HD033745.

#### P08.62

#### Characterization of post-transcriptional regulation of the human chromosome 21 transcriptome

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To characterize the post-transcriptional regulation of transcriptional units in human chromosome 21 (HSA21), we performed a sucrose gradient separation of RNA in 3 cell lines (GM06990, HeLaS3 and SKNAs). We generated fractions according to ribosome content to separate (i) RNA undergoing active translation (associated with at least 2 ribosomes) and (ii) a pool of all fractions representing total RNA. Each pool was hybridized to a 22bp resolution tiling array comprising the entire non-repetitive sequence of HSA21 (18 Mb).

We observed that approximately 5% of HSA21 is transcribed in each cell line, and a total of 8.5% is transcribed in at least 1 line. On average 51.6% of signals correspond to annotated regions, whereas the remaining have been previously referred to as Txfrags.

We performed RT-PCR (with RT-minus control) and/or 5' and 3' RACE for 100 random Txfrags, and sequenced positive bands. In all cases the sequences correspond to known exons elsewhere in the genome but also with high homology to HSA21. This strongly suggests that these signals result from cross-hybridisation.

To identify genes with significant levels of post-transcriptional regulation, we performed a paired-rank analysis of all detected exons. We observed that 86 out of 247 HSA21 genes demonstrate a significant shift in their rank distribution, suggesting post-transcriptional control. Our data suggest that 1) there is a considerable posttranscriptional control of gene expression and 2) a substantial fraction of Txfrags result from cross-hybridization with exons mapping elsewhere in the genome.

**P08.63****Prader-Willi Syndrome: Multiplex Ligation Dependent Probe Amplification Diagnosis (MLPA) and Microsatellites**

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Introduction: PWS is a neuroendocrine genetic disease, with a global frequency estimated between 1/10.000 - 1/25.000 livebirths. Clinical symptoms are characterized by hypotonia, hyporreflexia, lack of movements and deglutory alterations. Typical dysmorphologic and behavioural phenotype include hyperphagia-obesity, hypogonadism, hypogenitalism, mental retardation and bone maturation retardation.

Lack of paternal contribution results in PWS either by paternal deletion in 15q11-q13 region (70%) or from maternal disomy (25%). A few cases (2-5%) are due to imprinting center mutations.

Aims: We have performed a mutational screening of large deletions in 15q11-q13 region and the methylation analysis of *SNRPN* gene in 37 patients with PWS clinical features. The aim of this study is to confirm clinical diagnosis, to evaluate the main genetic alterations in our population and provide supportive genetic counselling to the families.

Material and Methods: Patients DNA was obtained by robotic extraction (EZ1, Qiagen). The MLPA technique was performed by the commercially available PWS Kit from MRC Holland. This kit allows large deletions screening in 15q11-q13 region genes in two PCR reactions and the methylation study of *SNRPN* by *Hhal* enzyme digestion. Analysis was performed with an ABI PRISM 310 sequencing analyzer (Applied Biosystems) and further data Excel normalization. In abnormal methylation cases microsatellites D15S10, D15S11, D15S113, D15S128, GABRA3, GABRB5 were studied.

Results and Conclusion: The MLPA genetic analysis has allowed PWS diagnostic confirmation in 16.2% patients with clinical criteria. Two deletions in 15q11-q13 region and 4 maternal uniparental disomy were found. We conclude that PWS is well characterized by the MLPA technique.

**P08.64****ProSeeK: A web server for MLPA probe design**

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The technological evolution of platforms for assessing genome-wide copy number imbalances has allowed the discovery of an unexpected amount of human sequence involved in duplications and deletions (termed copy number variants or CNVs). In terms of sequence coverage, this is the most important type of human variation identified so far and can make an important contribution to human diversity and disease susceptibility. While different methods exist to assess genome-wide changes in gene dosage at high-throughput, methods for validating these findings are tedious and expensive. Multiplex Ligation-dependent Probe Amplification (MLPA) is one of the available technologies, used even in diagnostic settings, to assess copy number variation in the human genome. One of the dull and time-consuming steps of MLPA is probe design step. Because of the multiplexing capacity and the required specificity, the oligonucleotides targeting specific regions have to meet a large number of requirements for an efficient design of the experiment. ProSeeK is designed to perform all steps automatically and to provide the best candidate probes for each individual assay. ProSeeK is integrated as a user-friendly web server to ensure portability.

**P08.65****The OpenArray™ platform: enabling high-throughput SNP genotyping and real-time PCR applications in nanoliter volumes**

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BioTrove has developed the OpenArray platform based on through-hole technology, a broadly applicable nanoliter fluidics technology for parallel and low-volume solution phase reactions. OpenArray plates are coated with hydrophilic coatings on the interior of each through hole and hydrophobic coatings on the exterior of the through-holes. This

enables OpenArray plates to hold solutions in the open through-holes via capillary action. The OpenArray plate consists of 3072 through-holes that can be loaded with reagents to perform individual 33 nL reactions for use in both real time PCR applications as well as endpoint genotyping applications. The unique configuration of the through-holes enables the researcher to interrogate a large number of nucleic acid samples against a large number of assays in a flexible, configurable format. By altering the number of assays or the number of samples the researcher can easily customize the OpenArray to meet their changing needs. Researchers using this technology benefit from the parallelism of microarrays and the data quality of solution phase reactions.

**P08.66****External cell control quantitative RT-PCR (eccPCR): A new technique for reliable detection of subtle changes in mRNA expression**

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Quantitative RT-PCR (qRT-PCR) is a widely used method to determine relative gene expression levels. Quantification of the observed expression levels becomes reliable after normalization to the expression of an internal standard gene. If the mRNA level of the standard gene is altered during the experiment, small changes of target mRNA levels can be especially difficult to detect. However, the expression of commonly used internal standard genes is often unstable, which considerably bias quantification.

To overcome the drawback of unstable internal standards, we developed a new method, called external cell control PCR (eccPCR). This method is based on the addition of control cells to the studied cells before RNA extraction and qRT-PCR. Only the control cells express the reference gene, while only the studied cells express the gene of interest. This technique controls all steps of sample preparation and overcomes the incertitude of normalization to internal standard genes.

We present the validation of the new method by detection of the changes of hSERCA3 mRNA expression in response to Na+-butyrate treatment of KATO-III gastric cancer cells using F4-6 mouse erythroleukemia cells as control and mPu.1 mRNA as the reference gene. In addition, we demonstrate the instability of the expression of a commonly used internal standard gene GAPDH by the eccPCR technique. The sensitivity analysis of the new method showed that a 1.5-fold gene expression level difference can be systematically detected with the eccPCR assay.

We conclude that eccPCR allows accurate quantification of small expression level differences of a gene of interest.

**P08.67****An integrated custom design tool for PCR resequencing**

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Increasing attention has been devoted to SNP discovery and genotyping in an effort to associate disease/phenotypes with gene variations and mutations and to determine evolutionary relationships, but reliable primer design and data analysis remain two of the major challenges to overcome. Development of a flexible design tool that allows researchers to select PCR-sequencing primers for different genomic targets with user-defined parameters would greatly facilitate resequencing.

In order to help the scientific community to better use PCR and CE-sequencing for SNP discovery, we released the VariantSEQR™ primer designs of 15K+ human genes to the public via NCBI's ProbeDB.

To improve upon this one-size-fits-all approach, we have developed an integrated web-based tool which incorporates target sequence selection/selection, primer design, and data analysis into a connected workflow. Users can choose genes, transcripts and other identifiers, select any region in the genome, or upload their own sequence as well as specify design parameters, e.g. amplicon length, primer Tm etc. The web interface then submits the job to a backend pipeline, which takes advantage of proprietary primer picking and predictive quality assurance processes that generated Applied Biosystem's VariantSEQR™ primers. All currently known SNP/MNP sites in the genomic

sequence are avoided during primer design. The resulting primers are then checked for genomic redundancy and the probability of success in PCR. An exhaustive search is performed to produce the optimal tiling of the amplicons covering the target region. Utilization of this primer design tool can substantially reduce the effort required to design and optimize robust resequencing primers.

#### P08.68

#### Prescreen for *RB1* mutation identification using high-resolution melting analysis

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Germ-line mutations in the *RB1* gene cause hereditary predisposition to retinoblastoma, a childhood eye cancer, but 90% of the tumors are caused by novel mutations. Since the *RB1* gene spans 180 kb and contains 27 exons, and *RB1* mutations have been observed in all 27 exons and promoter with few recurrent mutations, multiple modalities are required in addition to sequencing to attain high sensitivity for mutation detection. Amplicon melting with a saturating DNA intercalating dye was introduced as an attractive technique to genotype small sequence alterations. In order to investigate the efficiency of high-resolution melting analysis (HRM) in *RB1* mutation screening, we tested its sensitivity to detect known variants.

PCR products were prepared for routine sequencing. Resolight dye master mix was added post-PCR. Fluorescence melting curves were recorded using the LightCycler 480. All (10/10) heterozygous and 2/2 homozygous variants were readily discriminated from wild-type. A wide range of mutation types including heterozygous one base pair transitions and transversions and small deletions and insertions could be detected when compared with wild-type DNA. Our experiments showed that we were able to obtain different melting curves from wild-type DNA even for very low level mosaic variants which are detectable only by allele-specific PCR (AS-PCR) and not by sequencing. A good sensitivity can be expected when the assay is used to detect otherwise missed mosaic mutants. HRM analysis is a simple and rapid technique in prescreening of small sequence alterations in DNA diagnostics.

#### P08.69

#### The first serotonin receptor allelic variant database

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Serotonin (5-hydroxytryptamine, 5-HT) controls a variety of physiological functions. 5-HT receptors mediating serotonin action are divided into seven main classes (5-HT<sub>1</sub>R to 5-HT<sub>7</sub>R). A multitude of candidate gene screenings has been published during the last years. We have started to structure this information in the first serotonin receptor allelic variant database using LOVD (Leiden Open Source Variation Database). Up to now, the database comprises data of 5-HT<sub>3</sub> receptor subunits. To date, five different human subunits are known (5-HT<sub>3A,E</sub>), which are encoded by the serotonin receptor genes *HTR3A*, *HTR3B*, *HTR3C*, *HTR3D* and *HTR3E*. Different receptor subtypes seem to be involved in chemotherapy induced nausea and vomiting (CINV), irritable bowel syndrome (IBS) and psychiatric disorders. During the last years *HTR3* case-control and pharmacogenetic studies indicated that *HTR3A* and *HTR3B* polymorphisms may contribute to the etiology of psychiatric disorders and may predict CINV and medical treatment of psychiatric patients. Currently, the database is subdivided into five sub-databases, referring to the serotonin receptor genes. Within each sub-database we are collecting mutations, polymorphisms, demographic information as well as pharmacogenetic data. Every sub-database includes general information about the respective gene and is linked to other resources. The remote user is able to search the data and to submit new data. This central information pool should help clinicians as well as scientists to evaluate their findings and to use the information for subsequent studies. Data about functional consequences of variants will be integrated in future to enable specific drug design in the therapy of respective conditions.

#### P08.70

#### AutoSNP: an integrated graphical user platform of high-throughput SNP data analysis for disease gene mapping

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Single nucleotide polymorphisms (SNPs) have become most common used marker in current human genetic studies. Researchers normally need to select a set of SNPs from an enormous amount of SNP of candidate genes or regions. Moreover, current platforms of high-throughput SNP genotyping will produce huge amounts of genotyping data with different output format. It is a trivial and time-consuming work to pick up SNPs and process different kinds of data format for specific linkage or association analysis program.

To ease the task of managing high-throughput SNPs data from two main platforms-Illumina and Affymetrix assays, we generated a graphical user platform named "AutoSNP" by using Perl language. In this platform, user can select SNP data source and then convert SNP genotyping data into appropriate format for specific genetic analysis program. We integrated both linkage analysis programs (Allegro, GeneHunter, Linkage, Merlin, SuperLink and SimWalk2) and association analysis programs (FBAT, PLINK, HaploView, PHASE, and WHAP) in this platform. Moreover, this platform also allows users to connect to Ensembl variation database, NCBI SNP database, and the TAMAL (Technology And Money Are Limiting) database to query information from their SNPs list. Finally, we use a skin disease "primary cutaneous amyloidosis (PCA)" genotyping data as an example to demonstrate our platform.

#### P08.71

#### New Methods for Sizing Large DNA Fragments on Capillary Electrophoresis Instruments

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Applied Biosystems is continually developing new fragment analysis size standards to better support existing and new applications. Recent developments have focused on improving sizing precision and accuracy and expansion of the sizing range, using Applied Biosystems' 5th dye technology. We present details about developing methods to take advantage of a new high performance, 68 peak size standard and illustrate its versatility, accuracy, flexibility, and precision when used on various capillary electrophoresis instrument platforms, different polymers and capillary array lengths. Methods described include DNA fragment sizing up to 1200bp in applications such as AFLP, T-RFLP, VNTR, mutation screening, MLST, and BAC fingerprinting.

#### P08.72

#### A statistical approach for predicting the clinical type of SMA using MLPA

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Spinal muscular atrophy (SMA) is a common autosomal recessive disorder in humans, characterized by degeneration of the anterior horn cells of the spinal cord, causing symmetric proximal muscle weakness. SMA is classified in three clinical types, SMA I, SMA II, and SMA III, based on the severity of the symptoms and the age of onset. The purpose of this study was to build the prediction model evaluating the probability of a SMA type based on multiplex ligation-dependent probe amplification analysis with commercial probe mix (SALSA Probe Mix 021; MRC Holland). This mix contains 16 probes specific for the SMA critical region (5q12.2-5q13.3). Dosage ratio was chosen to form the basis of the analysis. We developed a multinomial logistic regression model based on the data of 65 SMA cases. Prediction model was constructed using all probes, and using stepwise probe selection algorithm by Akaike information criterion to remove irrelevant and redundant

probes while retaining or improving the predictive power. Validation of the model was performed using 10-fold cross-validation regime to avoid overfitting and assure statistical validity of results. The predictive model was evaluated on the basis of three performance measures: sensitivity, specificity, and the area under the receiver operating characteristics curve (Table 1). Proposed model confirm the known and indicate some novel patterns which may contribute to a better understanding of SMA.

Table 1. Performance measures.

	Model					
	Full			Stepwise		
	SMA I	SMA II	SMA III	SMA I	SMA II	SMA III
Sensitivity	0.471	0.679	0.700	0.571	0.719	0.737
Specificity	0.896	0.676	0.844	0.902	0.758	0.848
AUC	0.772	0.676	0.707	0.748	0.746	0.771

#### P08.73

#### SMRT arrays as a high resolution technique for detection of DNA microvariations

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Introduction: Nowadays, array CGH is one of the highest resolution techniques for genome-wide detection of chromosomal alterations. Genome screening using array CGH has great potential in the characterization of numerous 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.

The aim of this study was to show the utility of this SMRT arrays to provide precise information about the size and breakpoints of DNA copy number gains and losses.

Methods: We present a patient with microcephaly, epilepsy and mental retardation, with a female normal karyotype 46, XX. A SMRT array, (from Wan Lam Laboratory at the BC Cancer Research Centre) analysis was performed. Moreover, two MLPA kits (SALSA P096 and SALSA, from MRC-Holland) and fluorescent in situ hybridization (FISH) technique were carried out.

Results: The SMRT array analysis showed a 4p subtelomeric deletion of 1.25 Mb. MLPA study of the specific 4p16.3 subtelomeric region confirmed this microdeletion. Five of the sixteen specific probes of MLPA kit, located in the new defined WHSCR-2, were deleted. FISH assays showed a deleted pattern with the 4p subtelomeric probe but normal results were obtained when hybridization was performed with specific WHS probe.

Conclusions: The SMRT array study confirms the small deletion WHSCR-2 previously detected by MLPA and not detected by WHS FISH. SMRT array arises as an effective technique to detect DNA microvariations and provides more information about their size and precise breakpoints.

#### P08.74

#### Flexible and 640-multiplex array detection of nucleic acid variations

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DNA sequence variation detection is important in various biomedical applications such as gene identification of common diseases, disease diagnosis and -treatment, drug discovery and forensic analysis. Here, we describe an arrayed primer extension-based genotyping method (APEX-2) that allows multiplex DNA amplification (640-plex) and detection of single nucleotide polymorphism (SNP) or mutations on microarray through four-color single-base extension. The principle of multiplex PCR needs two oligonucleotides per SNP/mutation generating amplicons that contain the unknown base pair (studied position). The same oligonucleotides are used in the following step as attached single-base extension primers on a microarray. The call rate of 640-plex APEX-2 was 99.87%, the reproducibility was 99.92% and the coincidence between Illumina HumanCNV370-Duo v1.0 BeadChips and APEX-2 genotyping was 98.6%. The method described here may be very useful for a custom number of SNP- or mutation detection analysis, molecular diagnostics and in forensic analysis. APEX-2 has also the potential to fill the gap (up to 1500 positions) between expensive high-density platforms and low-multiplex level detection systems.

#### P08.75

#### Transplantation of amniotic fluid-derived stem cells in a rodent model of stroke

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The discovery of amniotic fluid (AF)-derived stem cells initiated a new and very promising field in stem cell research. These cells possess immune-privileged characteristics suitable for a successful transplantation. The purpose of this study was to reveal stemness of rat AF-derived cells and to test the effectiveness of transplantation of fresh as compared to cultured AF cells in a rodent model of stroke. Cells were characterized at day 25 of culture by immunohistochemical analysis to highlight the presence of embryonic stem cell markers. This analysis showed that cultured, i.e., adherent AF cells resembled a mesenchymal-like morphology and were positive for OCT4 and SSEA, typical embryonic stem cell markers. A parallel *in vivo* study subjected rats to middle cerebral artery (MCA) occlusion for 60 minutes and subsequently assigned to one of the following intravenous transplantation: i) freshly isolated AF cells; ii) cultured rat AF mesenchymal-like stem cells; iii) vehicle. Behavioral tests were performed prior to stroke surgery and repeated at day 2 post-stroke/post-transplantation to evaluate the functional consequences of the MCA occlusion and to quantify improvements in motor and cognitive function after transplantation. Transplantation of cultured, but not freshly isolated AF mesenchymal-like stem cells promoted significant recovery from stroke-induced motor and neurologic deficits compared to vehicle-infused stroke animals. The present study shows that cultured rat AF-derived cells are more therapeutically active than freshly isolated cells. The observed behavioral benefits demonstrate that AF stand as a highly potent alternative source of stem cells for therapeutic strategies in stroke and related neurological disorders.

#### P08.76

#### e-infrastructure for thalassaemia research network (Ithanet)

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The main objective of Ithanet (<http://www.ithanet.eu>) is to enhance the scientific potential of the haemoglobinopathies' research community using infrastructures and tools of European research networks. Ithanet aims to harmonise and develop these resources for the coordination of existing research activities as a base for future collaborative projects. The consortium comprises of all major European research institutions active in the field and a number of collaborating partner institutions from non-EU Mediterranean and Black-Sea countries. In total Ithanet associates 26 partners from 16 countries. In order to set-up and maintain interaction between the partners involved as well as to coordinate training and project management activities, Ithanet utilizes teleconferencing technology. Additionally Ithanet introduced media broadcasting and streaming technologies for conferences and teaching courses on haemoglobinopathies, contributing to the exchange and dissemination of good practices in the field of e-learning. Aiming to encourage a high level of interaction among members of the haemoglobinopathy community, a dedicated portal has been created (<http://portal.ithanet.eu>). Visitors to the Ithanet portal can quickly exchange information, share ideas, enhance the global awareness of pertinent issues, and help define critical areas of haemoglobinopathy treatment and research. The portal offers the latest news, techniques and information on haemoglobinopathies and also serves as a platform for the easy exchange of information and dialogues between interested groups, both patients and researchers. The portal is also used as a database of standard scientific protocols, methodologies, and repositories. It contains information on almost all Thalassaemia-related institutions and medical centres as well as patient and scientific societies.

**P08.77****Genomic regulation of gene-expression levels in the brain during human evolution**

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For the last few years, several groups have used microarrays to compare transcript levels in humans and non-human primates and have identified hundreds of candidate genes with expression changes in the human brain. Here, a multidisciplinary approach, including experimental techniques and bioinformatic analysis of genome sequence and gene-expression data, is proposed to obtain a more profound understanding of the potential causes and effects of these gene expression changes. Preliminary sequence comparisons have yielded examples of diverse types of mechanisms that could account for the observed differences in mRNA levels, such as multiple gene duplications, insertion of transposable elements in 3' UTR or upstream regions, and a single nucleotide mutation in the polyA signal. In addition, as an example of the proposed strategy, a global analysis of the regulatory sequences of the thrombospondin 4 (*THBS4*) gene has been carried out. This gene is involved in synapse formation and shows a six-fold up-regulation in humans that is specific of the forebrain. Interestingly, an excess of nucleotide changes and an Alu insertion were found in the *THBS4* promoter region in the human lineage. However, *in vitro* gene reporter assays indicated that the human and chimpanzee promoter fragments have similar transcriptional activity, and computational prediction of transcription factor binding sites suggests that *THBS4* up-regulation could be due to changes in trans-acting regulators. Overall, these results provide insights into the regulatory mechanisms acting at various levels and the evolutionary role of gene-expression regulation, and could help us to determine the molecular basis of human-specific traits.

**P08.78****Using genome-wide pathway analysis to unravel the etiology of a complex disease like type 2 diabetes**

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Several genome-wide association studies (GWAS) have recently been published on a variety of complex diseases. However, 99.9% of GWAS data is currently discarded and not analyzed to its full potential. In this study, we took a different approach and aimed to evaluate alternative methods of obtaining valuable information on disease etiology from GWAS data. We assessed whether we could detect overrepresented biological pathways in the GWAS datasets, by combining a network-based tool, 'Prioritizer', with a pathway-classification tool, 'PANTHER'.

As an example, we used publicly available data from two type 2 diabetes (T2D) GWAS; the Diabetes Genetics Initiative (DGI) and the Wellcome Trust Case Control Consortium (WTCCC). Of the 1,179 SNPs in the DGI dataset and the 1,712 SNPs in the WTCCC dataset that showed association with T2D with a p-value lower than 0.003, we were able to map 559 and 797 SNPs, respectively, to genomic loci that contained one or more genes. Prioritizer then selected the most promising gene(s) from each locus based on their functional interactions with genes on the other loci. We used PANTHER to assign each of the selected genes to a specific pathway and to test whether we saw more genes in each pathway than expected.

Our results showed that the 'inflammation mediated by chemokine and cytokine signaling' and 'Wnt signaling' pathways were consistently the most strongly overrepresented in the T2D data. By taking an alternative approach, we have shown it is possible to detect biological mechanisms in GWAS data.

**P08.79****Ultra-High-Throughput Sequencing in heterogeneous inherited heart disorders: a first experience in Hypertrophic Cardiomyopathy**

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During last decade, molecular genetics has provided new insights into the pathogenesis of inherited heart disorders and Hypertrophic Cardiomyopathy (HCM) represents the most common one (1/500). Genetic testing has a growing impact on the management of patients and their families. However, the extensive genetic heterogeneity of these disorders requires the use of high-throughput mutation detection strategies for clinical screening. We had developed a 30-Kb HCM-Custom-DNA-resequencing-array (HCM-RA) comprising all exons (n=160), splice-sites and 5'-UTR of 12 HCM genes. Although very efficient in detecting single nucleotide variants, this approach did not identify small indels, accounting for 14% of HCM mutations. Moreover, resequencing-arrays lack flexibility since gene additions requires a new design.

In order to overcome the shortcomings of microarray-resequencing we assessed the performance of the recently developed Ultra-High-Throughput-Sequencing (UHTS). We reanalysed these 12 genes in a total of 19 patients, hybridized previously on HCM-RA (8 positive-controls as a composite-pool and 11 DNA without mutation), in a single channel of a SOLEXA. Every single base of the sequence (570 fold coverage accounting for 24 alleles) was analyzed using a newly developed data analysis pipeline.

All the 8 control mutations and 18 SNPs previously identified by HCM-RA were also observed in UHTS. Furthermore sequence alterations and particularly indels were detected such as c.1028delC/p.Thr343fsX349, [c.2146-9C>A+c.2146-2delA], c.506-12delC in gene MYBPC3, c.53-15\_11delTTCTC in TNNT2. Although evolution and improvement in DNA target enrichment, specificity and data analysis are needed, UHTS holds considerable promises in mutation/variant analysis underlying highly heterogeneous or multigenic pathologic conditions in clinical practice as well.

**P08.80****Advantages of universal primers use in biochip analysis of thrombosis genes polymorphism**

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Genetic testing of inherited predisposition to frequent multifactor diseases needs new screening methods of DNA-polymorphism analysis. The most advanced approaches in genetic testing of multifactorial diseases usually include multi-locus DNA amplification. The multiplex PCR method could be significantly improved by means of the universal sequences incorporated into locus-specific primers. We have selected such DNA sequences and used them for two-step PCR genotyping of *F2*, *MTHFR*, *F5*, *PAI1*, *GPIIIa*, *FGB* polymorphism with the subsequent hybridization on oligonucleotide biochips. Use of this technique opens an ample opportunity for the fast and reliable detection of inherited thrombosis. The method could be easily adopted in any molecular diagnostic laboratory.

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**P08.81****The Human Variome Project - plans and progress****R. G. H. Cotton<sup>1,2</sup>:**<sup>1</sup>*Genomic Disorders Research Centre, Carlton South VIC, Australia, <sup>2</sup>Convenor, The Human Variome Project, Australia.*

The Human Variome Project (HVP; [www.humanvariomeproject.org](http://www.humanvariomeproject.org)) was created to coordinate and curate the collection of all genetic variation, its phenotype and associated disease(s). This is because lack of up-to-date, complete and correctly curated information can lead to excessive web searching, misdiagnosis and wastes valuable health-care funds.

Work to obviate this problem began more than a decade ago with the Human Genome Variation Society (HGVS; [www.hgvs.org](http://www.hgvs.org)) promoting collection and display of variants, producing recommendations and software.

At the launch of the HVP, 96 recommendations ([www.nature.com/ng/journal/v39/n4/pdf/ng2024.pdf](http://www.nature.com/ng/journal/v39/n4/pdf/ng2024.pdf)) were drawn up by over 50 world experts from over 20 countries to be implemented in the future. The task is large so countless people will need to be involved in a coordinated manner with specially developed tools and protocols.

What is needed is an automated, seamless system transferring clinical data (phenotype), genotype and pathological data to hospital records, as well as to databases curated by experts, in a de-identified and ethically acceptable way, initially to LSDBs and finally to central databases/browsers.

The International Society for Gastrointestinal Hereditary Tumours (InSiGHT; [www.insight-group.org](http://www.insight-group.org)) has volunteered to be a pilot for (a) collection of all mutations and phenotype for their four genes of interest and (b) from all countries.

Many components for this flow have already been developed, often multiple times around the world in an un-coordinated disconnected way. A planning meeting was held in May 25-29 2008 to review these and rationalise future planning ([www.humanvariomeproject.org/HVP2008/](http://www.humanvariomeproject.org/HVP2008/)).

**P08.82****Teststrip-based genotyping to assist in the prediction of anticoagulant dose requirement****H. Puehringer<sup>1</sup>, Q. Berisha<sup>2</sup>, G. Klose<sup>3</sup>, B. Schreyer<sup>3</sup>, W. Krugluger<sup>2</sup>, R. Loretz<sup>3</sup>, C. Oberkanins<sup>1</sup>:**<sup>1</sup>*ViennaLab Diagnostics GmbH, Vienna, Austria, <sup>2</sup>Department of Clinical Chemistry, Rudolfsstiftung Hospital, Vienna, Austria, <sup>3</sup>Clinical Haemostaseology, Westpfalz-Klinikum GmbH, Kaiserslautern, Germany.*

Coumarin derivatives, such as warfarin and phenprocoumon, are the most widespread oral anticoagulant drugs for the prevention and treatment of arterial and venous thromboembolic disorders. However, these vitamin K antagonists have a narrow therapeutic range and a wide interindividual variability in dose requirement. Despite adjustment for clinical variables, adverse events are frequently encountered during the initial phase of therapy. Genetic polymorphisms in the drug-targeted vitamin K epoxide reductase complex 1 (VKORC1) and in the drug metabolizing enzyme CYP2C9 have been reported to account for the majority of variations in the therapeutic response to warfarin.

We have developed a genetic test (StripAssay) for the simultaneous detection of two VKORC1 polymorphisms (-1639G>A, 3730G>A) and the functionally defective CYP2C9 variants \*2 and \*3 determined by 430C>T and 1075A>C. Preliminary data from our ongoing clinical study, to date including 130 patients treated with phenprocoumon (Marcumar™), allowed a classification of high, intermediate and low dose responders according to VKORC1 and CYP2C9 genotypes. The stable dosage required for therapeutic anticoagulation was considerably lower in carriers of a combined VKORC1 -1639A and CYP2C9 \*2 or \*3 genotype compared to carriers of a single variation or wildtype alleles. The VKORC1 3730G>A polymorphism seemed to have no additional predictive power for phenprocoumon dose variability.

The new diagnostic assay and the results obtained during our study will assist clinicians to achieve a safer and more individualized anticoagulant therapy.

**P08.83****eSensor® genotyping test for CYP2C9, CYP4F2 and VKORC1 polymorphisms associated with warfarin sensitivity****W. A. Coty<sup>1</sup>, M. R. Reed<sup>1</sup>, A. R. Jacobs<sup>1</sup>, P. Naranatt<sup>1</sup>, R. Hubert<sup>1</sup>, S. Panuganti<sup>1</sup>, Z. Wang<sup>1</sup>, V. Headley<sup>1</sup>, Y. Liu<sup>1</sup>, M. Abedi<sup>1</sup>, G. R. Gust<sup>1</sup>, K. Olszewski<sup>1</sup>, D. Canfield<sup>1</sup>, Osmetech Molecular Diagnostics, Pasadena, CA, United States.**

We have developed an eSensor® test to genotype 8 CYP450 2C9 polymorphisms known to affect enzyme activity (2C9 \*2, \*3, \*5, \*6, \*11, \*14, \*15 and \*16), as well as the VKORC1 -1639G>A promoter polymorphism associated with warfarin sensitivity and the CYP450 4F2 rs2108622 (V433M) polymorphism recently found to correlate with warfarin dose. After multiplex PCR and exonuclease digestion, genotyping is performed using the eSensor® cartridge and XT-8 instrument within 35 minutes for up to 24 samples. In a reproducibility study performed with 20 genomic DNA samples and 3 plasmid controls (n = 345 tests), 100% first-pass call rate and agreement with DNA sequencing were obtained. A method comparison study with 105 genomic DNA samples extracted from blood gave 100% call rate and agreement with DNA sequencing after re-testing of no-call samples, as did an additional cohort of 145 genomic DNA samples from saliva and cell lines. The assay gave 100% first-pass call rate and agreement with DNA sequencing using input genomic DNA amounts between 10 and 1,000 ng per PCR. Testing of ethnic panels from the Coriell Cell Repository revealed elevated allele frequencies for 2C9 \*5 (3%) and \*11 (7.6%) in the African-American panel (n=33), and 2C9 \*14 (2.2%) in the Gujarathi Indian panel (n=90). Initial feasibility has been demonstrated for PCR amplification directly from whole blood samples, with no interference observed from serum albumin, IgG, bilirubin, hemoglobin, triglycerides or excess EDTA.

**P08.84****A multiplex detection assay for Warfarin dosing using Single Base Extension****A. J. Rai<sup>1</sup>, N. Udar<sup>2</sup>, C. T. Yu<sup>1</sup>, M. Fleischer<sup>1</sup>:**<sup>1</sup>*Memorial Sloan Kettering Cancer Center, New York, NY, United States, <sup>2</sup>Beckman Coulter, Fullerton, CA, United States.*

Thromboembolic events in "at risk" individuals can be prevented by anti coagulation drug therapy. Warfarin is a commonly used anticoagulant prescribed to over one million patients in the US (2006). Individuals vary greatly in their response to warfarin therapy. This difference has a genetic basis. Two genes: cytochrome P450 2C9 (2C9) and vitamin K epoxide reductase subunit protein 1 (VKORC1) have been reported to account for 60% of these differences. There are two clinically important alleles of 2C9(\*2 and \*3) and one of VKORC1 (-1639 G>A). We designed a multiplex assay for the simultaneous detection of these three alleles in a single reaction. Our assay entails a multiplex PCR amplification of the target gene fragments followed by a multiplex single base extension reaction. The single base extension reaction incorporates the target nucleotide which has a fluorescent tag attached to it. This tag is detected by separation on a capillary electrophoresis platform. The entire assay can be performed within an eight hour day by a single technologist with minimal hands-on effort. We observe 100% concordance on twenty samples when our assay is compared to traditional DNA sequencing. We have optimized this assay for high-throughput screening of patient samples, allowing for analysis of two 96-well plates on a single overnight run. Our multiplex SNP panel can be used as a stand-alone test for patients starting warfarin therapy, or its results can be combined in an algorithm with additional parameters (e.g. weight, age, sex, etc.) to provide dosing recommendations for initial warfarin administration.

**P08.85****Whole Genome Amplification (WGA) in forensic SNP profiling****I. Pietrangeli<sup>1</sup>, C. Martone<sup>1</sup>, E. Giardina<sup>1</sup>, I. m. Predazzi<sup>1</sup>, P. Marsala<sup>2</sup>, I. gabriele<sup>3</sup>, c. pipolo<sup>2</sup>, o. ricci<sup>2</sup>, G. Solla<sup>3</sup>, A. Spinella<sup>3</sup>, G. Novelli<sup>1,4</sup>:**<sup>1</sup>*Centre of Excellence for Genomic Risk Assessment in Multifactorial and Complex Diseases, School of Medicine, Tor Vergata University of Rome, Rome, Italy, <sup>2</sup>Direzione Centrale Anticrimine, Servizio di Polizia Scientifica, Rome, Italy,*<sup>3</sup>*Direzione Centrale Anticrimine, Servizio di Polizia Scientifica, Rome, Italy, Rome, Italy, <sup>4</sup>Division of Cardiovascular Medicine, Department of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, United States.*

Whole genome amplification (WGA) is a technique developed for genetic analysis to obtain sufficient amount of DNA from small pools of cells or even single cells.

Presently, the usefulness of WGA in STR-based forensic analysis is limited because of allelic dropout (ADO) and bias in peak area ratios observed in low copy number (LCN) templates. Sequence polymorphisms (SNPs) are more sensible than length-polymorphisms (STRs) and less prone to ADO (allelic dropout) when WGA is applied. Thus, we recently validated a panel of TaqMan SNP assays selected to show an high sensibility in LCN templates. Here we evaluated the performance of multiple displacement amplification (MDA) applied to our optimized SNP assays starting from small amounts of genomic DNA (gDNA). A set of 100 samples were analyzed for 21 SNPs and a total of 1 ng, 100 pg and 10 pg of genomic DNA of each sample was used as template for the MDA. Concordance and amplification failure (AF) between gDNA and wgaDNA were extremely robust (100%) when WGA was performed on 1 ng and 100 pg of gDNA, whilst the concordance decreased to 99.2% for samples amplified from 10 pg of gDNA. The absence of a full concordance for 10 pg samples is referred as ADO occurring when a gDNA heterozygote genotype is scored as homozygote but it should not lead to mis-typing if only heterozygous genotypes are considered. The robustness of WGA applied to specifically selected SNP assays should suggest a reconsideration of WGA for forensic SNP profiling.

#### P08.86

#### Comparison of genotyping consistency between genomic and whole-genome amplified DNA using the Illumina GoldenGate and Infinium-II assays

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High-throughput SNP genotyping has become an important research strategy in human genetics. Although most genotyping assays require minimal amounts of DNA, repeated use often leads to depletion of the samples. To address this problem whole-genome-amplification (wga) technologies have been developed and are meanwhile commercially available. Albeit the amplification seems to be mostly successful, it is controversially discussed whether the wgaDNA represents an exact copy of the genomic DNA (gDNA). In the present study, we aimed to assess the genotyping consistency between 45 wgaDNAs (generated using the REPLI-g DNA Amplification Kit, Qiagen, Hilden) and their corresponding gDNA samples. The gDNAs were of different age and quality. To compare genotype consistency 20 high-quality sample pairs were genotyped using Illumina's HumanHap550V3 BeadChips. (565.000 SNPs). 25 sample pairs of different DNA quality were genotyped for 384 SNPs using Illumina's GoldenGate assay. All samples genotyped on the HumanHap550V3 performed well, with call rates >99%. The average consistency between gDNA and wgaDNA was 99.99% when comparing SNPs successfully genotyped in the corresponding samples. Of the 25 sample pairs genotyped with the GoldenGate assay, 22 performed well with call rates >99% (gDNA) and >98% (wgaDNA). Genotype consistency was 100% for corresponding samples. The remaining 3 sample pairs showed noticeably worse results with an average genotype call frequency of 99.8% (gDNA) versus 60.1% (wgaDNA) and a genotype consistency of only 89%. Possible explanations for the observed discrepancies include the age of gDNA, the extraction method as well as the presence of unknown inhibitors interfering with the amplification process.

#### P08.87

#### Comprehensive Desktop Software for Next Generation Sequencing Applications

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Next Generation sequencing technologies provide the data generation tools for many large scale molecular biology applications including whole genome sequencing/resequencing, polymorphism detection, as well as gene expression and regulation. The cost effectiveness of these technologies makes them accessible to virtually any researcher. One of the lagging issues, however, has to do with the handling of the large quantities of data generated and gaining access to the tools required to analyze the data. To provide users with the ability to take advantage of the next-gen revolution, DNASTAR has developed fully scalable software capable of processing a wide range of resequenc-

ing and whole genome projects on a desktop computer. The software is fully compatible with Sanger, Roche 454, Illumina and ABI SOLiD sequence platforms. We will provide workflow examples of assembly projects along with the use of its companion, SeqMan Pro for finishing, analyzing and annotating whole genome assemblies. We will also present a workflow for using the software in digital gene expression applications comparing data from microarrays to data generated by next generation sequencing instruments.

### P09. Genetic counselling, education, genetic services, and public policy

#### P09.01

#### Diagnostic and clinical validations in DNA-diagnostic laboratories: a BRCA example

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Clinical validations are used to assess the clinical sensitivity and specificity of genetic testing within DNA diagnostic laboratories and are an important instrument to monitor and further improve reliability and efficiency of molecular testing. However, for most indications the clinical information available to DNA diagnostic laboratories is too scarce to determine the a priori chance of a positive test result. In order to assess the sensitivity and specificity of genetic testing, we started with a so called diagnostic validation, in which the robustness of the method(s) to analyze a single gene or a set of genes for a genetic disease was determined. The parameter used is the mutation detection ratio (MDR) which is defined as the proportion of mutation-positive results. By comparing the MDR of a genetic test at different time intervals (e.g. years), the robustness of this test can be judged. This robustness depends on changes and variations that occur within a method (with or without preceding analytical validation) as well as method performance by different operators. Decrease of the MDR of a genetic test over time, should lead to critical evaluation of the different laboratory processes and assessment of putative alterations in the a priori risk of the diagnostic requests.

An example: The efficiency of BRCA1 and BRCA2 mutation analysis has decreased within years. To determine the cause of this decrease, e.g. whether this is due to technical issues or solely to an altered referral policy, we performed both a diagnostic validation as well as a clinical validation.

#### P09.02

#### Expanded newborn screening: Challenges for the provision of pre-newborn screening care

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Historically, newborn screening (NBS) aimed to identify serious childhood disorders for which treatment was available to reduce morbidity and mortality. Limited attention to pre-screening information provision was justified by the severity and treatability of the conditions screened. Yet technological advances allow NBS programs to identify disorders for which the promise of clinical benefit is uncertain as well as an array of "incidental" findings (benign variants, carrier status results). Given the contested value of such results, commentators argue that limited attention to pre-screening care is no longer justifiable. This paper reports survey data on attitudes and practices of a cross-sectional stratified random sample of five health care professional (HCP) groups in Ontario that are involved in prenatal care and/or care of newborns in the first days of life (obstetricians, midwives, nurses, family physicians, pediatricians). The majority of HCPs surveyed (68%) believe it is their responsibility to provide information about NBS to parents prior to the heel prick test. However, as many as 48% of these providers report that they do not consistently or usually do so. This paper explores the role of provider type, practice barriers (e.g. insufficient time, training, compensation) as well as knowledge and confidence of NBS in explaining the discrepancy between perceived professional responsibility and actual professional practice. Thus, while most HCPs perceive

a responsibility to provide information about NBS to parents prior to the heelprick, many challenges face the provision of this increasingly important pre-screening care.

#### P09.03

##### **Increasing research through collaboration**

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One goal of lay advocacy groups is to encourage research into the condition of interest. However, advocacy groups face some unique challenges in the achievement of this goal. (1) The pool of potential research participants and funding opportunities are limited by the relative rarity of the condition. (2) Many conditions have multisystem involvement, meaning that specialists from multiple disciplines must be involved in the research. (3) For many conditions, the natural history is still unknown. However, studies delineating the phenotype of a condition are particularly challenging, as such studies require a methodical clinical assessments of every system, ideally performed by the same group of investigators.

The relationship between the Chromosome 18 Registry and Research Society and the Chromosome 18 Clinical Research Center is unique in that the Registry is affiliated with only one research center. This differs from the more traditional paradigm in which an advocacy group grants smaller amounts of funding to multiple investigators at different institutions.

In our experience, this relationship has been critical in addressing the above challenges. (1) The collaborative effort provides the Research Center both funding and a large pool of potential research participants. (2) The establishment of the Research Center in one institution allows for recruitment of multiple disciplines within the institution to aid in study design and execution. This paradigm also eliminates barriers to the sharing of data and samples between investigators. (3) The same investigators perform evaluations on patients using the same methods and tools, ensuring that the clinical data is reliable.

#### P09.04

##### **geneticsmadeeasy.com**

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genetics made easy.com is a free informative web site about human genetics, with an average at this moment of 490.868 visits for year. It works as an excellent resource for clinician an other healthcare professionals in order to complement the genetic counseling as well as for general population and couple wanting to have a baby as the web site presents important information that they should know. Them terms used in the web site are clear, easy to understand, and are supported by static illustrations as well as by flash animations.

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? (assisted reproduction techniques) - Origin of hereditary disorders - Prenatal Diagnosis techniques - Gene therapy - Cloning and stem cells - Questions - Links of interest - Further reading - Foreword

#### P09.05

##### **The international multidisciplinary Community Genetics Network**

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The International Multidisciplinary Community Genetics Network is an E-mail network aiming to facilitate communication among those working in the field of community genetics. This includes researchers, health professionals and others interested in genetic screening, genetic education, access and quality of genetic services or preconception care, genetics in primary care, genetic registries, genetics of disadvantaged populations, public consultation or epidemiological, economic,

psychosocial, ethical and legal issues.

The core activity is a monthly newsletter with a list of references to recent scientific papers of members and a continuously updated list of upcoming meetings. This allows a rapid spread of information among members, which is advantageous to the authors of papers or organizers of meetings, but also to readers who want to stay tuned on what others are doing. In addition members may present information on new activities in the newsletter, and publish calls for information (e.g. about validated research questionnaires or participants for a particular study).

The Network was launched at the ESHG annual meeting in Nice last year. Nine months later (mid February 2008) the Network has 245 members in 45 countries worldwide, growing at a pace of 25-30 new members each month. Just over 50% of the membership comes from Europe (20 countries). The country with the largest number of members is the USA. Updated numbers will be presented at the ESHG annual meeting in Barcelona.

Those who want to have more information or want to become a member and obtain the newsletter, should send an E-mail to [commgenet@gmail.com](mailto:commgenet@gmail.com) .

#### P09.06

##### **Mainstreaming community genomics: the ECOGENE-21 initiative**

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ECOGENE-21 is a transdisciplinary initiative, driven by the specific needs of communities. ECOGENE-21 specifically refers to human communities, a cohesive social entity of individuals or families sharing the same environment and ecosystem within the context of the larger society. ECOGENE-21 aims to understand the role of genetic health determinants and new genetic technologies in global health issues, and integrate this knowledge into a comprehensive framework to improve health at the level of human communities. The aims are to: (1) develop, validate, apply and replicate cost-effective genome-based technologies and screening strategies to prevent Mendelian diseases at the community level; (2) generate, validate, apply and evaluate technologies and strategies in different communities of the world for the prevention and treatment of common diseases, based on the model of lipid disorders, the metabolic syndrome and associated risk of type 2 diabetes and cardiovascular risk factors; (3) develop the expertise and platforms needed to generate, apply, validate and replicate new knowledge issued from community genomics, population genomics, pharmacogenomics, nutrigenomics, epigenomics and environmental genomics research. Over a 5-year timeframe, ECOGENE-21 will deliver, apply and disseminate new applicable, cost-effective and exportable vanguard technologies, strategies and concepts for disease prevention in human ecosystems, as well as innovative knowledge transfer and training strategies, tools, platforms and expertise. The ECOGENE-21 framework and results obtained to date in the French-Canadian founder population based on analytic, clinical and public health validity and utility criteria will be presented. ECOGENE-21 is supported by the Canadian Institutes of Health Research (#CTP-82941).

#### P09.07

##### **It's not just about competence: Why identifying organisational practice is an important step in developing a high quality genetic service**

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While there have been numerous efforts to develop competences in genetics for non-genetic health professionals to support their practice, there has been minimal research to identify other factors that influence this role. As part of our study to examine how midwives provide a genetic service in Victoria, Australia, we identified the barriers and facilitators of this practice, using a mixed methods approach. Qualitative data were collected from nine focus groups with midwives (n=50) and two focus groups with experts in genetics (n=10), as well as 12 interviews with managers of hospital maternity services. Transcripts

were analysed, major themes identified and data triangulated. These findings were validated with quantitative data collected from midwives responding to a state-wide survey (n=317, response rate 45.4%). Our findings indicate that there is variation in the genetic practice of midwives. While each midwife's practice is contingent on their individual competence, the scope of their practice is heavily influenced by the organisation in which they work. For example, each hospital provides specific guidelines on what information needs to be collected to determine genetic risk, and what genetic tests should be discussed with each pregnant woman. These findings suggest that programs that only target midwifery competence will not be enough to effect practice change. Instead, for midwives to provide an effective and high quality genetic service, a two pronged approach targeting midwifery competence and addressing organisational policy is required.

#### P09.08

#### **Genetic counseling for newborn hearing screening in Nagano, Japan**

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Congenital deafness is a disorder with high prevalence (1/1,000 birth) and that requires early diagnosis and medical intervention to minimize the morbidity of the affected infant. Since 50% of congenital deafness is caused by genetic defect and clinical phenotype and prognosis in part depend on genetic background, genetic testing for affected infants can provide useful information. In Japan, a newborn hearing screening has started as a government project in 2000. In Nagano prefecture of Japan (population 2.2 million), a screening program has started in 2002. In 2007, under the collaboration and coordination among medical-, educational- and administrative staffs, the Support Center for Children with Hearing Loss has opened to support both affected children and their parents. Division of Clinical and Molecular Genetics in Shinshu University Hospital also collaborates with this center as a provider of genetic counseling. Since 70% of genetic congenital deafness is an autosomal recessive disorder, most parents of affected children have normal hearing, thus do not consider the possibility of genetic causes on their affected children. This also suggests that the burden of patients could be more intense when they know the diagnosis of their children.

We performed a questionnaire-based survey to clarify psychological impact of parents and what they needed during hearing screening, before and during genetic testing, and after being informed a diagnosis. Results of this survey clearly indicated the importance of genetic counseling during screening program and intense collaboration between the Center and community medical staffs such as public health nurses.

#### P09.09

#### **Development of a set of core competences in genetics for health professionals in Europe**

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The increasing use of genomics within a wide range of health care settings requires health professionals to develop relevant expertise in genetics to practise appropriately. There is a need for common minimum standards of competence in genetics for health professionals in Europe. However, there are significant differences in professional education and practice across Europe and setting common curricula is not practical. It was agreed by the Expert group of the EuroGentest project Unit 6 that a pragmatic solution would be to describe and agree, by consensus, a set of core competences that could apply to health professionals in Europe, whatever their national setting.

The core competences were based upon existing frameworks, modified for the European context. They relate to i) professionals whose specialisation is in genetics and ii) professionals who are generalists or specialise in an area of health care other than genetics. Recommendations have been based on work that was developed in consultation with the particular group of professionals involved. The goal of this

work is not to be prescriptive, but to provide frameworks that can be adapted to national and professional need. Curricula for a range of health professionals, both pre-registration and post-registration, can be built or modified using the competences.

In this paper, the process of development, the competences and suggested learning outcomes will be discussed. The competences and a background document can be viewed on the website of the EuroGentest project [<http://www.eurogentest.org/>]. The project team invites feedback.

#### P09.10

#### **Genetic Counsellor : News from France**

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The Professional Master « Genetic Counselling and Predictive Medicine » made possible the education of the first French Genetic Counsellors, since 2005. Two years later, there are 34 graduated, 23 of them have a job and 4 of them have a job offer.

Thanks to the data from the French Association of the Genetic Counsellors; the AFCG (Association Française des Conseillers en Génétique), we suggest to present the interdisciplinary recruitment, the job progression in France, and the way managed to its valorisation.

We will specify the annual number of new graduated, and the Genetic Counsellors location in France and in a couple of countries. Then, we will insist on the processing action for a national, European, and international integration of these new French professionals.

#### P09.11

#### **Genetics counseling:misdirection and misuse in some developing countries**

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Practically genetics counselling began with Eugenic movement around 1910 in England,USA and some western countries with very negative and destructive effects on health and integrity of humankind. Fortunately ,the misuse of genetics was officially abandoned in 1944 though practically persisted up to 1960s.

Medical Genetics counselling based on dignity and equality was introduced by Sheldon Reed in 1944 and became an obligate branch of modern clinical and medical genetics settings.

In Islamic countries Genetics counselling was introduced by the author in 1968 based on the same principles thought by my mentor professors Reed,Vogel and Fuhrmann, and considering all of the humanitarian,ethical,moral ,and religion issues concerning the clients.Since 1968 i have consulted more than 17000 couples,majority seeking consanguineous marriage. Only less than 2% of these young healthy couples seeking marriage actually needed karyotyping.

Unfortunately,in the last several years in some developing countries,genetics counselling has been misunderstood and misused due to financial interests of private centers.It is quite wrong but popular practice to order karyotyping for every marriage or every disease that carries the label of being due to genetics.s disease.I have seen many examples of ordering karyotyping for Duchene muscular dystrophy,thalassemia,hemophilia,phenylketonuria and cystic fibrosis .

Based on these facts I am proposing a universal medical guideline for every Genetics counselling center and every genetics counsellor based on medical,ethical,humanitarian ,and religion principles so that the needy clients could not be misused and financially and morally exploited.

#### P09.12

#### **Cystic fibrosis cascade carrier testing in Victoria, Australia: an audit of clinical service**

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In Victoria, Australia, carrier testing for cystic fibrosis (CF) is performed by a single state-wide laboratory and counselling service. CF carrier

testing is offered free of charge to the relatives of babies diagnosed with CF through the newborn screening program. Although cascade testing is known to detect carriers of CF, its effectiveness has been questioned, in our clinical service and elsewhere, because most babies with CF are born to couples who do not have a family history; the uptake of cascade testing following a child's diagnosis of CF by newborn screening has not been previously reported.

We investigated CF cascade testing in the families of Victorian children with CF. We studied the uptake of cascade carrier testing by examining 53 pedigrees of newborns diagnosed with CF between 2001 and 2004, and performing data linkage to the laboratory database records. The uptake of carrier testing amongst adult first and second degree relatives was 16% (190/1160). Parents were the most likely relatives to have been tested, followed by aunts/uncles, then grandparents.

We conclude that cascade testing, as currently offered, is not an effective strategy for detecting carriers for CF. An alternative is to offer population-based screening and this occurs in some countries but with quite variable uptake reported. Factors influencing uptake of CF carrier testing need to be explored. We are now conducting an evaluation of the barriers and facilitators to cascade testing to inform both clinical service delivery and population-based CF screening programs.

#### P09.13

##### Facing choices about genetic testing and assisted reproduction: developing an integrated care pathway to help health professionals meet the needs of infertile men with Cystic Fibrosis (CF) and congenital bilateral absence of the vas deferens (CBAVD)

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**Background** The choices offered by assisted reproductive technologies (ART) and preimplantation genetic diagnosis (PGD) mean that genetics services need to be better integrated into the care pathway of infertile men with CF/CBAVD. This study explored how multidisciplinary healthcare professionals in one UK NHS Trust believe genetics services can be integrated into a common care pathway.

**Methods** Three focus groups were convened with health professionals involved in the care of men with CF/CBAVD (representing genetics, adult respiratory medicine, paediatrics, assisted reproduction and urology). The first two groups (5 & 7 participants respectively) explored awareness of genetics, ART and PGD. Concerns and training needs were identified. Following patient interviews, a final focus group was convened to identify a common care pathway.

**Results** Much uncertainty existed amongst non-genetics specialisms regarding the role of genetics services in the CF/CBAVD care pathway. A lack of confidence was evident when talking about the genetics of CF/CBAVD, ART and PGD. Common concerns related to when to raise these issues and how to deal with parental pressure. Participants felt that although the in-depth discussion of these issues should be the main responsibility of the genetics service, their own basic knowledge could be enhanced.

**Conclusions** There is a need for improved information exchange about the genetics of CF/CBAVD and assisted reproductive options between specialisms. Genetics services can improve awareness and become better integrated into the existing care pathway by facilitating professional workshops and training sessions, and producing leaflets and referral guidelines. An integrated care pathway is being developed.

#### P09.14

##### Challenges in establishing a population carrier screening program for cystic fibrosis

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Cystic fibrosis is the commonest life shortening autosomal recessive condition among Caucasians due to mutations in the *CFTR* gene. The carrier frequency in this population is 4% with a disease incidence of 1:2,500. Carriers are healthy and the majority of people are unaware

of their carrier status. More than 95% of babies born with CF have no family history of CF. Most parents who have a child with CF elect to utilise reproductive technology for subsequent pregnancies by either prenatal diagnosis (PND) or pre-implantation genetic diagnosis (PGD). The US National Institutes of Health and American College of Obstetricians and Gynecologists recommend offering CF carrier testing to all couples.

We have introduced a fee paying prenatal screening program, initially offered through obstetricians in the private sector. Sampling is by cheek brush, testing for the twelve most common severe mutations in *CFTR*. In the first two years of the program 2975 people were screened of whom 64 were carriers (1:46) and 6 carrier couples (~1:500) were identified all of whom chose either PND if pregnant or PGD if non-pregnant at the time of testing.

The challenges and difficulties faced include difficulties of educating health care professionals and the public about CF, the requirement for sample re-collection in 2.2% of patients screened, the correct collection procedure, patient anxiety regarding not being offered screening until pregnant, reluctance of health professional to offer screening primarily due to time constraints and equity of care when screening is currently only offered in the private health sector.

#### P09.15

##### EuroGentest Medical Genetics Quality Assurance Database

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The outcome of genetic testing has a great impact on the life of patients and their entourage. The quality of genetic testing is thus of utmost importance. In July 2007, EuroGentest released a European Quality Assurance (QAU) database in collaboration with Orphanet, with reliable public information about the quality systems of laboratories offering medical genetic testing (<http://www.eurogentest.org/web/qa/basic.xhtml>). The database contains at present data from about 230 laboratories of 32 countries.

These data comprise besides laboratory contact details and a link to the Orphanet laboratory page, information about the quality manager, the accreditation status with a link to the accreditation scope if applicable, and about participation in genetic EQA schemes.

During 2004 and 2005, 152 of the 230 laboratories (66%) participated together in 87 different EQA schemes; mainly for CF testing (51% of the EQA participating laboratories), DNA sequencing (34%) and FRAXA (31%). Of the laboratories participating in EQA, 28% are also accredited according to standards ISO 17025 or ISO 15189. Another 23% of laboratories are preparing for accreditation in the near future. The EuroGentest QAU database allows consumers (laboratories, doctors, etc) to identify a laboratory with a quality system for a particular diagnostic test. It benefits laboratories by encouraging and providing recognition of their investment in QAU as well as by giving them a better informed choice for referral of tests.

The database will be continually updated and participation in the database is freely open to all European laboratories offering human medical genetic testing. For more information, please contact [QAusurvey@eurogentest.org](mailto:QAusurvey@eurogentest.org).

#### P09.16

##### Definitions of genetic testing in European and other national legislation (EuroGentest WP3.4)

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We are comparing definitions of genetic testing among legislation from European institutions and individual Member States, as well that produced in the USA, Canada, Japan and Australia.

Investigation is based on documents collected from websites and databases, including governmental sources, as well as that gathered within WP3.1 and the series European Ethical-Legal Papers, being published by EuroGentest Unit 6.

We checked the present status and validity of all legislation used: only those still in force will be analyzed and compared; when validity check is currently not possible, due to unavailability or language incompat-

ibility, they will be noted as "unknown".

Preliminary results (16 laws) come from 7 countries (Belgium, Hungary, Portugal; Australia, USA, Canada and Japan): 6 are compulsory and 10 soft laws.

Definitions of genetic testing in these documents vary significantly in length and comprehensiveness, depending on their general purpose and aims; jurisdiction source is also variable: general discrimination (Belgium and Canada), genetic information and discrimination in employment (USA), health care (Hungary), general privacy and confidentiality (Australia), health and genetic information (Portugal).

The expected results are an up-to-date and easy-to-search definitions database, focusing on legislation of European and other countries, with annotations regarding relevancy and reach of that legislation (compulsory or only soft law; source, and to which jurisdiction(s) does it apply; etc.).

Then, definitions of genetic testing in European and other legislation may be compared among countries and jurisdictions. This might be helpful for legislators and policy makers, the various professionals involved in genetic testing and the general public.

#### P09.17

#### What do judges need to know about human genetics? Judicial science education in the US

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The importance of genetics has grown in the past decades not only among other biological sciences but also throughout society in general. However knowledge about the subject lags. Efforts have been devised to fill this gap in high schools, in medical education and in the general public. We'd report here on judicial education programs as conceived and produced by the Advanced Science and Technology Adjudication Resource Center (ASTAR) in collaboration with the University of North Carolina in Chapel Hill.

ASTAR is a congressionally mandated educational effort with the mission of training the US judiciary in scientific matters in order to enhance scientific knowledge in US courts. Through the use of lectures and hands-on instruction a cadre of resource judges has been trained to serve at their home jurisdictions as resource persons who can offer consultation and expertise when cases involving scientific and technical matters arise.

We present aspects of the curriculum, which range from intense "basic science boot camps" to mock "adjudication clinics" and plenary discussions moderated by judges and scientists. We focus specifically on human genetics as this subject plays an increasing role in diverse court cases. The examples include genetics of addiction, cancer genetics, genetically engineered crops, inborn errors of metabolism and genetic discrimination. Our educational objective is to promote case management and settlement in controversies in which novel scientific evidence is likely to be introduced.

#### P09.18

#### Etnicity and Genetics: a sensitive issue

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In our multicultural society registration of ethnicity is not allowed by law. We here present six "ethnicity issues": in genetic counselling and diagnostic testing, population screening programs, association studies and pharmacogenetics, respectively.

- Testing for new mutations in the BRCA-1 gene is time-consuming. Patients with an Ashkenazi background have different mutations than other ethnic groups and therefore, it would be advantageous to know the ethnic background of patients to improve efficiency of mutation analysis.
- In prenatal screening for Down syndrome, background values for Beta-hCG and PAPP-A differ between Afro-Caribbean and West-European women.
- In neonatal screening 16 diseases are tested in all 180.000 newborns in the Netherlands each year, including sickle cell anaemia, while only 40.000 have an increased risk for that disorder.
- Consanguineous marriages are common in people originating from

North Africa, because of social advantages. The increased risk for autosomal recessive diseases in their offspring is mostly not known in the future parents.

- In association studies results can often not be reproduced by additional studies. Ethnicity is an important cause.
- Finally, ethnicity is an issue in pharmacogenetics, for instance in prescribing beta-blockers for heart failure in African-Americans. It is difficult to handle the issue of ethnicity in genetics correctly and little is done to change that attitude. Meanwhile, this attitude may be harmful for the patient and science in general.

#### P09.19

#### A comparison of criteria for clinical validity and utility in various national and international frameworks

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CAPABILITY is a 3-year model project developed jointly by Unit 3 (Clinical Genetics, Community Genetics and Public Health) and Unit 6 (Education) of the Network of Excellence (EuroGentest) and by leading experts from Argentina, Egypt and South Africa. CAPABILITY's overall objectives are to contribute to the efforts to establish and sustain a worldwide harmonisation process for quality standards for the integration of genetic test/genomic knowledge applications into practice and prevention and to serve as a model project for successful, sustainable collaboration between EU research centres and centres from developing countries.

In recent years a great deal of attention has been paid at national and international levels, to develop policies in the field of provision of clinical genetic testing services. The topic has been tackled by several different national and international organisations, each taking different approaches, depending on their primary objective. In various frameworks different groups addressed the determination of criteria for clinical validity and utility of genetic testing. As a preliminary outline in the context of CAPABILITY, this review aims to present the various frameworks and to draw a comparison of the different approaches.

#### P09.20

#### The cystic fibrosis external quality assessment scheme, monitoring the quality of laboratory performance

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Given the potential serious health consequences of genetic test results, mechanisms should be in place to assure the quality of the tests and the interpretation of the data.

In this regard, the International Organization for Standardization ISO 15189 (5.6.4) and the Organisation for Economic Co-operation and Development (OECD) Guidelines for quality assurance in molecular genetic testing (2.C), contain requirements and recommendations for laboratories to participate in external quality assessment schemes (EQA). Participation in EQA schemes is useful not only to the laboratory, as a key element of its quality assurance processes, but also as a quality indicator to monitor the improvement of laboratory performance.

The Cystic Fibrosis (CF) Network has been providing EQA since 1996 and has performance data from more than 10 years. This study focuses on a group of about 100 molecular genetics laboratories that participated in the CF EQA scheme for each of the previous three years. Reporting the correct genotype, provision of appropriate interpretation and additional elements required by ISO 15189 such as unique identification of the patient and sample type, are compared over these three years. In addition, comparable cases and samples with identical genotypes were included during the three-year period, which enabled

us to analyse performance of a laboratory more specifically and to survey if individual comments, provided by the assessors, were taken into account by the laboratory.

Regular participation contributes to continuous improvement and monitoring of internal quality in laboratory performance. Moreover, it is a relevant tool for assessors to educate laboratories.

#### P09.21

#### Fetal alcohol syndrome among grade-one children in the Northern Cape Province of South Africa: prevalence and risk factors

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Objective: To describe prevalence, characteristics and risk factors for fetal alcohol syndrome (FAS) and partial FAS among children attending grade one in the Northern Cape Province of South Africa.

Design: Cross-sectional study using a two-tiered method for active ascertainment of FAS/partial FAS cases. This comprised screening of growth parameters, and then diagnostic assessment for screen-positive children using clinical and neuro-cognitive assessments, and maternal history of drinking during pregnancy. Mothers or care-givers of children with FAS were interviewed, as well as matched controls.

Setting: Primary schools in De Aar (8) and Upington (15).

Subjects: Grade one pupils in 2001 (De Aar, n=536) and 2002 (Upington, n=1299).

Outcome measures: FAS or partial FAS.

Results: Prevalence of FAS/partial FAS was high: 64/536 (119.4/1000, 95% CI=93.2-149.9) in De Aar, and 97/1299 (74.7/1000, 95% CI=61.0-90.3) in Upington. Overall, 67.2 per 1000 children (95% CI=56.2-79.7) had full FAS features. Growth retardation was also common in this population: 66.6% (1181/1774) were underweight, 48.3% (858/1776) stunted and 15.1% had a head circumference <2S.D. for age. Interviews with cases and controls showed that mothers of children with FAS were less likely to have fulltime employment or have attended secondary school. These women also had lower body mass index and about 80% currently smoked. Over two-thirds of all pregnancies were unplanned.

Conclusions: Nearly one in ten pupils has FAS/partial FAS, with the rate in De Aar the highest yet described in South Africa. The epidemiological features described are important for designing essential preventive interventions.

#### P09.22

#### A study into the knowledge of inherited metabolic disorders among patients and parents in the Irish population

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Galactosaemia and Maple Syrup Urine Disease (MSUD) are recessively inherited conditions detected by newborn screening in Ireland. Patients are treated at one centre and genetic information is provided by the specialist team. We assessed knowledge among parents and patients to see whether referral for formal genetic counselling would be beneficial, using a questionnaire including 4 demographic, 8 knowledge, 2 information and 5 disease impact questions. 27 families with galactosaemia and 10 with MSUD were interviewed in clinic. All parents of children with galactosaemia and MSUD answered >75% of questions correctly, but there were misunderstandings about the risk or implications of carrier status. There was a significant difference in knowledge between ethnicities. Adult patients with galactosaemia had more misunderstandings in relation to inheritance, recurrence risks and carrier status than their parents. 83% of study participants requested more information about their condition and its transmission. 40% of affected adults with galactosaemia identified a need to meet others with the same condition. While parents of children with MSUD or galactosaemia are well informed, the majority expressed a wish for more information. Adult patients themselves and parents from an Irish Travelling back-

ground could especially benefit from further genetic counselling.

#### P09.23

#### Molecular testing for rare genetic disorders in Europe: hype or hope?

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Currently, there are huge differences between various countries in accessibility, price and quality of molecular diagnostic testing. The bottlenecks include the vast number of genetic diseases (> 1000), the low number of samples per disease, the nature of the disease mutation often being a private mutation, the high cost of testing and lack of reimbursement by governments and insurance companies, and the lack of an international organised network of diagnostic labs combining their portfolio of tests. All these bottlenecks impair a cost-effective and reliable diagnostic service, thereby holding molecular testing in many countries in a preclinical era. However, the quality, accessibility and cost-effectiveness of diagnostic tests for rare genetic disorders could be substantially improved by the creation of an international network of diagnostic labs combining their portfolio of tests and exchanging samples for rare genetic disorders. The first network of diagnostic labs offering genetic tests internationally was incorporated four years ago, and is called GENDIA (for GENetic DIagnostic Network). It consists of "referral labs" sending samples to GENDIA, "test labs" testing samples they receive from GENDIA, and a central GENDIA lab coordinating the network. Currently more than 2.000 different genetic tests are available through GENDIA. Such international network of genetic diagnostic labs results in greater access to a large spectrum of genetic tests performed with higher quality at lower cost.

#### P09.24

#### What is the impact of genetic counseling and prenatal diagnosis in genetic diseases prevention in an Arab Muslim population?

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Tunisians are mostly of Arab and Berber origin, and nearly all are Muslim. Consanguineous marriages are prevalent. Tunisia is different from its neighbouring countries in its accomplishments towards women's rights. The nuptial evolution toward a belated marriage model reduced the progeny. Contraception is encouraged and abortion is legal in case of medical or acute social problem. Genetic disorders are common in Tunisia; more than 70% of the population are educated and most are receptive to health guidelines. Genetic counselling (GC) and prenatal diagnosis (PND) are performed as medical facilities in a limited number of genetic centres. To evaluate the real impact of GC and PND on genetic diseases prevention, we surveyed during three years 2862 couples /families who were referred to our center. We evaluated the impact of GC on parents' attitude by analyzing the occurrence of pregnancies and the acceptance of prenatal screening and PND. Parameters were correlated to parents' age, socioeconomic situation, and education level; and to the disease. Our results showed that people in Tunisia ask for GC and follow it in most cases. The response quality is variable depending on the patients' education and their socio-economic class. This is in some ways different from other Arab countries with similar cultural and religious backgrounds, probably due to social and legislative differences. At present, GC and PND seems to be the method of choice for prevention of genetic diseases in Tunisia and such services should be developed as a priority despite the financial costs of such a programme..

#### P09.25

#### Exploring the views of European clinical genetic professionals on new international recommendations for genetic counselling related to genetic testing

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*EuroGentest: The main goal of the EuroGentest Network of Excellence is to improve and harmonise the quality of genetic testing in Europe.*

Formulation of quality standards includes those for genetic counselling associated with genetic testing, and is the focus of this study.

**Genetic Counselling Guidelines related to Genetic Testing:** The recommendations for genetic counselling in connection with different testing situations aim to establish minimal criteria for genetic counselling and to improve patient's understanding of the results and consequences of genetic testing. These guidelines were formulated through analysis of published guidelines and synthesis during two workshops with experts from genetic practice and research. Comments from ESHG members and national societies have been received, and endorsement by ESHG Board will now be sought.

**Study description:** This study aims to investigate how colleagues in the clinical genetics community view these new professional guidelines, and to identify any attitudinal, cultural, or practical barriers (professional/societal, economic etc) which may inhibit them following these guidelines in practice. Furthermore, the best ways for dissemination of guidelines will be discussed, and potential barriers to this identified.

**Study method:** Guidelines and questionnaire surveys will be distributed to self-selected delegates at the ESHG conference and selected genetics centres in countries across Europe. Approximately 100 individuals involved in providing genetic counselling will be recruited.

**Open invitation to obtain copy of guidelines and participate in study:** ESHG delegates are invited to visit our poster to receive a copy of the recommendations and to register an interest in participating in the study.

#### P09.26

##### Importance of database reviewing before prenatal diagnosis

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Cystic Fibrosis (CF) is an autosomal recessive disorder having a frequency of 1:4000 in our population. So far, we had study more than 2000 samples, including about 1500 from neonatal CF screening since 1999.

Several families with affected members or carriers asked us for genetic counselling.

In 2000, four brothers and sisters of a CF patient's mother were analysed for the F508del mutation. One of them was found to be a carrier of this mutation (heterozygote) while the others were non CF carriers. Then, the carrier's partner was screened for 95% of the CF causing mutations in our population using the DGGE-GC Clamp method. We observed an anomalous DGGE pattern in exon 11, and by direct sequencing we confirmed that it was the change I539T, previously described as a CF mutation. We informed the couple that the risk for having affected children was ¼.

Eight years later, in 2008, the obstetrician called us for a prenatal diagnosis of this couple. We reviewed the database and there were some changes: in 2002 this "mutation" had been subjected to functional studies in mammalian cells showing that I539T not only does not decrease the CFTR activity, but in fact increased its activity. Therefore, the obstetrician and the couple were informed that prenatal diagnosis is not necessary.

Preconceptional carrier screening allows couples at risk to arrive at informed reproductive decisions. Still, the information given to them may have to be updated at the moment of choice between different reproductive options.

#### P09.27

##### Results of Genetic Counselling and molecular genetic testing of severe monogenic disorders in Hungary between 1993 and 2006.

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During the genetic counselling of the severe monogenic genetic disorders it is very important to establish the exact etiological diagnosis. The molecular genetic examinations play outstanding role in this diagnostic procedure. Molecular genetic diagnosis provides effective possibility for prevention of serious genetic disorders by prenatal diagnosis and possible termination of pregnancy whenever treatment of diseases is unavailable. Moreover, genetic results provide accurate

differential diagnosis and proper medical care for this patients. Molecular genetic examinations on the following severe genetic disorders have been performed in our laboratory during the last 13 years: Spinal Muscular Atrophy - in 78 families with 98 prenatal diagnosis; FRAXA - in 38 families with 2 prenatal diagnosis; Charcot-Marie-Tooth disease type 1A - in 14 families; Duchenne/Becker Muscular Dystrophy - in 12 families; Congenital Myasthenic Syndrome - in 1 family; Facioscapulohumeral Muscular Dystrophy - in 2 families; Limb Girdle Muscular Dystrophy - in 2 families; Angelman syndrome in 5 families. The detailed results will be reported in our presentation.

By performing the molecular genetic analysis effective diagnosis and correct genetic counselling was established in our genetic unit.

#### P09.28

##### Inventory and classification of genetic diseases: a new service of Orphanet

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Several nomenclatures and classifications of genetic diseases have been developed since 1990, which overlap at the level of cellular models, molecular assays and genomic testing, as biological and medical researchers rarely communicate among each others. Genomics represent one end of the scale of granularity. Electronic Health Record Systems reside in medicine and form the other end of the scale. Both have to be able to exchange information, for instance to make genetic diseases visible in the health care system, but also to allow communication within the community and permit the interfacing of different databases. Nomenclature of genetic diseases has evolved a lot during the past years, but no one knows how to establish the catalogue of human genetic diseases as a definition of what is a disease is lacking. To overcome this difficulty, Orphanet ([www.orpha.net](http://www.orpha.net)) has established a database of phenotypes and of genes with the possibility to query by disease name, by gene name or symbol or by sign. Every phenotype is also classified in the multiple possible classification systems to allow an understanding of its physiopathology or its range of expression. Every phenotype has a unique identifier which will remain stable. Currently over 4,500 phenotypes are classified and indexed. This new service, available since March 2008, is expected to provide a bridge between clinicians and biologists for a mutual benefit.

#### P09.29

##### Collaboration is key in the preliminary efforts of genetic education for health professionals in Chile for the welfare of patients with genetic diseases

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With all clinical geneticists in Chile(25), in 2003 we implemented a website for clinical consultations that serve urban and rural hospitals; Telegenética in Spain began simultaneously. Interchange of technical support financed by Agencia de Cooperación Iberoamericana allowed the Chilean website ([www.teledismorfologia.cl](http://www.teledismorfologia.cl)). We have made preliminary efforts to use this site as a learning tool for postgraduate students in Clinical Genetics from Latin America.

During 2005 we developed a pilot course "Online Education in Clinical Genetics for Health Professionals" for those working in the only population-based registry of congenital birth defects in Chile. In 2006 and 2007 (57 and 71% MDs, 0 and 13% foreigners, respectively) we offered the program through [www.medichi.cl](http://www.medichi.cl), a net of digital learning from Faculty of Medicine Universidad de Chile, and are planning the third version 2008.

The website and the course should improve care for patients and their families by helping primary health care professionals understand ge-

netic influences on illness, and recognize and manage more effectively the most common genetic problems, with improving identification and description of children affected by birth defects.

Another challenge is facilitating the creation of support groups of rare diseases at a national level, demonstrating the needs of patients and groups; implementing access to the media, policymakers and the health systems. We struggle for the connection and interchange through an Ibero-American network of these alliances in each country.

Chilean population is 15 million, infant mortality 8 per 1,000 live births, congenital malformations the second leading cause (33.6%). Abortion is prohibited by law.

#### P09.30

##### Estimation of patient's understanding of genetic information received at genetic counselling. Results of pilot study.

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The pilot study of the 71 families with hereditary disease in a child or in one of spouses was carried out. It has been shown, that about 72% of respondents (51/71) have completely understood the genetic information. In group of respondents with low (to 5%) repeated genetic risk correctly named value and category of risk 83% and 63% accordingly. The appreciable part of respondents in this group (36%) has overestimated the genetic risk. About 70% of respondents with high repeated risk could correctly specify the value, and about 75% of them - a category of risk; 24% of them underestimated the risk. The full satisfaction with the results of consultation was expressed by 76 % (54/71) of respondents. A rate of coincidence of expectations of a family before consultation and the results of consultation was estimated. The respondent's satisfaction with the results of consultation was higher if their expectations were justified at least partially. This factor didn't influence the understanding of the information

#### P09.31

##### Capacity building for the transfer of genetic/genomic knowledge into practice and prevention: The CAPABILITY international collaborative network

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The number of genetic tests is growing each year and increasing knowledge about gene-disease associations will lead to new opportunities to apply genetic/genomic knowledge in practice and prevention. Before genetic tests are introduced into general practice the benefits of their use must be evaluated. Worldwide, health care systems are facing the same challenges: 1) The need to develop an evidence-based evaluation process for genetic tests or other applications of genomic knowledge in transition from research into practice. 2) The need for capacity building to enable health care systems to make effective use of genetic/genomic applications with proven clinical utility.

CAPABILITY is a 3-year model project developed by the Network of Excellence: Genetic Testing in Europe - Network for test development, harmonization, validation and standardization of services (EuroGentest) and by leading experts from: Argentina, Egypt and South Africa, the latter being currently engaged in major development projects to integrate genetic services in primary care and prevention in their countries.

CAPABILITY will:

- develop an analytic framework for evidence-based genetic test evaluation,
- identify priorities for capacity building by a systematic needs assessment survey and
- validate the project's approach by means of demonstration projects in Argentina, Egypt, Germany and South Africa.

CAPABILITY's overall objectives are to contribute to the efforts to establish and sustain a worldwide harmonisation process for quality standards for the integration of genetic test applications into practice and prevention.

#### P09.32

##### The lay people attitude towards predictive genetic testing in Russia

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1400 Russian adults aged from 20 to 65 (73% women) were investigated through a specially developed questionnaire about their base knowledge and attitude towards predictive genetic testing. 66% of respondents indicated they would prefer to know about their future diseases; the share of respondents expressing the positive attitude towards predictive testing increased (up to 85%) when people were told that the necessary prophylaxis will be in principle available. 89% of respondents mentioned they are likely change their life style (give up with pernicious habits, keep to a diet or take medication) if they would be diagnosed as being at a high risk of a disease; the proportion of women was higher if compared to men (91% vs 81%).

Among the main reasons which can force people to undergo predictive genetic testing for common diseases the people's concerns about their health status (39%) and practitioner's recommendation (23%) have been denoted; at the same time the more detailed information about genetic testing and curiosity have been accounting for 18 and 16% respectively, while 4% of respondents would follow the family member's or friend's advice.

When people were asked to range the particular common traits to which genetic testing could be mainly useful, they indicated oncological (17%), cardiovascular diseases (16%) and diabetes mellitus (11%) as the most advisable.

In general, the overestimation of expectations related to genetic testing for disease predisposition is obvious in lay people in Russia. The positive attitude for predictive genetic testing did not depend from the level of education, while the awareness about emerging genetic technologies did.

#### P09.33

##### Risky genes, risky trust? Framing risk through trust and credibility markers in direct-to-consumer online genetic tests

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This study looks at internet direct-to-consumer (DTC) advertising for genetic testing to assess the ways in which genetic risk information is framed to consumers, and examine the strategies employed to establish trust and credibility in this context. Representations of benefits and risks on 22 company websites were coded and themes were developed across advertisements for tests across a cross-section of health conditions. Two strategies were most frequently used by companies to frame risk: underlining the basis of the condition, often with genetic determinist and essentialist undertones, and stressing the commonality of the conditions. Major credibility and trust markers employed included indications of organizational professional accreditation and expertise (often through credentials of company executives and staff). The DTC ads examined provided limited, vague or inaccurate information about disease etiology and promoted tests for use in broader at-risk populations than is normally indicated in clinical practice. Implications of these trends for Canadian consumers and clinicians and for public policy are discussed.

#### P09.34

##### Information on genetic testing in Europe: new services from Orphanet

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Genetic tests are offered internationally, through both public and private sectors. Doctors and biologists need to know which tests are available, where they are offered and whether the identified laboratories meet quality standards.

To fulfil this need, ten years ago, www.orpha.net started to set up a database of medical laboratories in the field of rare diseases. The data collection covered one country in 1997, 15 in 2003, 26 in 2006 and reaches 36 countries in 2008. This effort was possible thanks to collaboration with EuroGentest NoE and resources from the EC DG for

public health. Orphanet is accessed daily by over 20,000 users, 20% of whom are looking for information on genetic testing. Currently, the database includes data from 1,233 laboratories offering 16,336 tests for 1,504 diseases. Data collection is done at country level through partnerships with key leaders in the field. Information is updated yearly through an online questionnaire prefilled with existing information. In March 2008, Orphanet launched a new website version to improve its user-friendliness and to provide expanded information. The new services include a possibility to query by gene in addition to the traditional query by disease name. It also includes the possibility to query by broad category of diseases, as all published classifications of rare diseases have been introduced in the database. Information on laboratories was enriched with quality management data, collected and validated by the QAU database team of EuroGentest. The new Orphanet website also includes information on networks in which laboratories participate.

#### P09.35

#### **Genetic testing: can a consensus definition be achieved? A survey of EuroGentest participants and website registered users (WP3.4)**

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EuroGentest Unit 3 sent a questionnaire to all participants, experts, advisory board and users of its website, to assess the need and the feasibility of achieving a consensus definition of genetic testing or whether this should be context-dependant.

A total of 126 questionnaires were received, from 32 countries: 58 EuroGentest participants, experts and advisory board members, and 68 registered users of its website. Among all, 44 were physicians (24 clinical geneticists, 20 other), 82 non-physicians (58 geneticists, 24 other professionals - lawyers, ethicists, etc.).

The need for a consensus definition was acknowledged by all groups, though all were also less enthusiastic about the possibility of attaining one (non-physician, non-geneticist professionals felt less that need, and were more pessimistic about reaching it). Clinical geneticists were the most supportive for context-dependent definitions. Human geneticists uphold that it should be best reserved for DNA/cytogenetic testing, whereas other groups supported a wider definition. Medical applications (and scientific research for many) should always be covered, but most believed non-medical applications (paternity testing, criminal identification) should not. Monogenic and susceptibility testing gathered consensus, but about 50% thought non-disease linked polymorphisms should be excluded. For 3/4, somatic mutations belonged within the definition. DNA, chromosomes and gene products were almost unanimously seen within its scope; clinical pathology, imaging/physiologic tests, physical exams and family history were perceived by ~50% of clinical geneticists also as covered, but less so by the other groups.

At issue, may be more the distinction between the definition of genetic-material testing and of genetic information.

#### P09.36

#### **IronXS: an Australian high school screening program for haemochromatosis**

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New research suggests that population genetic screening for hereditary haemochromatosis (HH), an easily preventable iron overload condition, should be reconsidered.<sup>1</sup> In the HaemScreen workplace-based study<sup>2</sup> we found high uptake of screening (>90%) for people attending the information session, but only 6% of those eligible actually attended. This led us to consider other strategies for offering screening.

We report our first year of data from ironXS, a screening program for the C282Y *HFE* mutation in high schools. A DVD-based information session was presented to 3638 year 10 and year 11 students at 19 schools. 1533 students had parental consent to participate (42% of eligible students; males:females = 45:55; mean age=15.7yr) of whom 1359 chose testing (37.4% overall uptake; 89% of attenders). This revealed 7 C282Y homozygotes (1 in 194), who were invited to clinic for genetic counselling, and 148 C282Y heterozygotes (1 in 9), who received their result in the mail. Students completed a baseline questionnaire (Q1). More than 90% of students answered all the knowledge questions correctly. A second questionnaire (Q2) was sent one month after results were received to all homozygotes and heterozygotes and a sample of wild-types (74% response rate). Knowledge retention was generally very high. There were no significant differences in general health perception scores, risk perception and anxiety levels between the three groups. Follow-up will include Q3 at 12 months and interviews with students, parents and teachers. We aim to screen 9000 students in total.

<sup>1</sup> Allen et al NEJM 2008, <sup>2</sup>Delatycki et al Lancet 2005

#### P09.37

#### **Genetic Counselling challenges with a family with HDL2: From the bedside to the bench and back to the bedside**

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Huntington disease (HD) is a late-onset genetic disease characterised by involuntary movements and progressive mental deterioration. The condition was thought to be caused only by a CAG-repeat sequence in the huntingtin gene on chromosome 4. In 2001 however it was found that an African American family, presenting with similar symptoms to HD and no expansion in the HD gene, had a mutation in the junctophilin-3 gene on chromosome 16. This condition has been named Huntington disease like-2 (HDL2) and until recently, had only ever been reported to be associated with individuals of Black African ethnicity.

In 2007 one family of mixed ancestry from the Western Cape in SA was identified with HDL2. This finding came about as a result of a pilot study of 63 persons with clinical symptoms of HD who do not carry the HD-causing expansion. Two individuals tested positive for HDL2 ; one that was referred for diagnostic testing in 1995 and the other in 2005. It was then found that in 2006, a research project had been initiated in another research laboratory, to study this family in-depth and blood samples had been collected from a number of additional family members. In addition, the mother of an eleven year old child of one of the HDL2 patients, described as a "difficult child", had recently requested genetic testing.

A clear distinction between clinical practice and research is not always possible and geneticists often find themselves with discordant responsibilities to different members of the same family.

#### P09.38

#### **The Human Genetics Historical Library**

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Although digitisation of historical sources represents a satisfactory solution to the accessing of many historic scientific journals and related works, for books, apart from a few classics, it is no substitute for availability of the printed works; yet many institutional and personal collections across the world are being dispersed or even destroyed.

The Human Genetics Historical Library represents an international resource, curated by Cardiff University Library Special Collections, that aims to create a definitive physical collection of books on or related to human genetics, covering the 20th century. Initiated in 2004, it now contains over 2000 volumes, almost all donated by individual workers or institutions.

The detailed catalogue records are becoming available online via Cardiff University Library catalogue (Voyager), <http://library.cardiff.ac.uk/>, while an interim listing is available on the project website ([www.genmedhist.info/HumanHistLib](http://www.genmedhist.info/HumanHistLib)), supported by Wellcome Trust. The books themselves are available for consultation. Donations are welcome.

The Library, as it grows further, will become a valuable historical resource in documenting the progress of human and medical genetics during the course of the past century.

**P09.39****Establishing a cardiogenetic service in Southern Sweden**

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<sup>1</sup>Dept of Clinical Genetics, Lund, Sweden, <sup>2</sup>Dept of Cardiology, Lund, Sweden. The genetic causes of several familial cardiac arrhythmias have been established in recent years, and thus the request for molecular genetic analyses as well as genetic counselling for these families has emerged.

The multidisciplinary cardiogenetic service in the South Swedish Health Care region was initiated in 2005, and the network now comprises cardiologists, pediatric cardiologists, clinical geneticists, and specialists in pathology and forensic medicine, respectively. Much effort is concentrated towards education of the stakeholders and on drawing up guidelines, whereby more uniform information and clinical management of at-risk family members is possible. So far, two guidelines have been completed, i. e., regarding families with an increased risk of hypertrophic cardiomyopathy (HCM) and long QT syndrome (LQTS), respectively.

We have adopted the model developed for our cancer genetic service, i.e., most families are seen by a clinical geneticist together with a cardiologist. This gives the possibility of discussing most aspects of the disease, although no clinical examination or investigation is performed. Until December 2007, 28 families have been counselled in the cardiogenetic clinic, the majority having familial HCM (15 families). 3 families had LQTS, and the rest had various diagnoses. All HCM-patients with a positive DNA analysis have had various mutations in *MYBPC3*. In LQTS, only families with a known mutation have, so far, been referred to the cardiogenetic clinic.

The request for genetic counselling in hereditary cardiovascular diseases is increasing, and establishing multidisciplinary networks in this field in our health care region is essential for high quality care and cost-effectiveness.

**P09.40****Should preconception genetic testing of infertile couples be any less rigorous than for gamete and embryo donors? A case report**

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A 36 year-old man presenting with primary male infertility (oligozoospermia) underwent in-vitro fertilisation treatment with intracytoplasmic sperm injection and transfer of two embryos resulting in healthy twins. The couple had surplus embryos cryopreserved, which they subsequently elected to donate to our embryo donation programme. To comply with Human Fertilisation and Embryology Authority (HFEA) Code of Practice for UK gamete and embryo donors, both partners underwent appropriate genetic screening tests, including karyotyping, which wasn't considered necessary when the couple were first evaluated at their local hospital. The man carried a 13;14 balanced Robertsonian translocation and was referred for genetic counselling.

Structural chromosome abnormalities cause infertility in both men and women, and many fertility centres have experienced similar cases. Owing to the increased incidence of balanced translocations among infertile people compared with the general population it is prudent to routinely offer preconception genetic counselling and karyotype analysis to couples with infertility. Such measures may improve (i) infertility diagnosis, (ii) follow-up treatment, (iii) risk assessment for future children and (iv) pregnancy management. Furthermore, appropriate genetic testing and evaluation may reduce numbers of failed IVF cycles, saving patients financial, physical and emotional costs. Current UK regulations require rigorous screening (including karyotyping) for gamete and embryo donors. However, routine karyotyping of couples with infertility is not UK standard practice and, this represents an inequality in patient care. Routine karyotyping should be offered to all couples presenting with infertility allowing them to make informed choices before undertaking the large investment required for assisted reproduction.

**P09.41****Analysis of Machado-Joseph Disease Pedigrees: Risk Assessment and Patterns of Segregation in Azorean Families**

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Machado-Joseph disease (MJD) is an autosomal dominant neurodegenerative disorder of late onset (mean 40.5 yrs), whose causative mutation is a CAG expansion in the *ATXN3* gene, at 14q32.1. MJD presents clinical heterogeneity, with differences in onset between series of patients being reported. Since in MJD risk assessment is complicated by age dependent penetrance, these differences will have an impact in risk calculation for at-risk individuals who choose not to take the genetic test. In the Azores 32 extended MJD families were identified; in Flores Island the disease reaches the highest worldwide value of prevalence (1:103). Segregation ratio distortion (SRD) could be one of the factors behind high values of prevalence. The availability of an extended genealogical database for the affected Azorean families, associated to the thorough follow-up of patients provided the background to conduct a study aiming: a) to provide age dependent risk data, with impact on Genetic Counselling (GC); b) to analyse segregation patterns of normal and expanded *ATXN3* alleles. For risk assessment, the probability of gene expression, using onset data from 176 Azorean patients, was calculated; a Bayesian method to compute the probability of heterozygosity if asymptomatic at different ages was applied. Sixty-two sibships were selected for segregation analysis (330 meioses). This analysis produced mendelian ratios, not supporting the presence of SRD for expanded alleles. Globally, results obtained will allow a higher accuracy of risk assessment, an essential component of GC, which is critical for the decision making of at risk individuals, namely for reproductive choices.

**P09.42****Science and Technology in the Muslim World: The Challenges**

**A. I. Al-Aqeel:**

Riyadh Military Hospital/ King Faisal Specialist Hospital, Riyadh, Saudi Arabia. Muslim nations must take a big leap forward in developing science and technology to catch up with the rest of the world. But to flourish, science and technology need a cultural base that can only be acquired by science education, with an ethical background, and by undertaking research programmes. This effort requires that the mentality of political leaders must change to show more of a commitment to science between the 57 Islamic countries.

Saudi Arabia, Qatar and Kuwait spend about 0.2% of their gross domestic product (GDP) on science - less than one-tenth of the developed country average of 2.3%. The Emir of Qatar has created an endowment that generates millions of dollars in research funding every year. Saudi Arabia is making a slow start, having approved a new national science and technology development plan in 2002. Both Saudi Arabia and Kuwait are each investing around \$2 billion in higher education institutes that include research centers.

Inherited Genetic diseases are prevalent in the Muslim World. We will address the preventive health aspects of genetic problems in the Muslim world and provide guidelines to prioritize preventive strategies. Applications of various novel genetic techniques such as comprehensive neonatal screening, high throughput heterozygote detection, and pre implantation genetic diagnosis are discussed.

**In conclusion:** from the various genetic techniques available, each country should adopt strategies most suitable to its genetic needs and should prioritize the programs to be used in prevention, in the presence of the challenges of having resources and expertise, among others.

**P09.43****Bases for genetic counselling in melanoma: benefits of specific surveillance programme**

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Introduction: *CDKN2A* and *CDK4* are two major susceptibility genes for melanoma (MM). Other genes involved in melanoma susceptibility

are *MC1R* and *c9orf14*.

**Objectives:** To describe the prevalence of germ line mutations in *CDKN2A* and *CDK4* in familial melanoma; to evaluate the modifying effect of *MC1R* polymorphism and *c9orf14* variants; to describe a specific surveillance programme.

**Subjects:** 70 families with at least 2 cases of melanoma (1 of 8; 17 of 3 and 49 of 2) were included.

**Methods:** Exon 1alpha, 1beta, 2, 3 and IVS2-105, -34G>T at the *CDKN2A* promoter region and EXON 2 from *CDK4* were studied by PCR-SSCP analysis and sequencing; *MC1R* was studied by sequencing and *c9orf14* variants by SSCP analyses.

**Results:** *CDKN2A* mutations were detected in 24% of families being more frequent in families with multiple cases ( $p<0.05$ ). The polymorphism A148T was present in 18% of families, while this polymorphism is only present in 7.2% of sporadic MM ( $p=0.02$ ). *MC1R* variants are associated with melanoma risk, increased number of primaries and younger age of onset. 268 individuals from 133 families were included in a specific dermatological surveillance programme including total body photography with digital dermoscopy, total body exploration with digital dermoscopy or total body exploration with dermoscopy according to the phenotype of the patient. This specific programme allows the early diagnosis of 92 new melanomas from 407 excised lesions in a mean follow-up of 44 months.

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#### P09.44

##### The European Molecular Genetics Quality Network (EMQN)

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European Molecular Genetics Quality Network (EMQN), NGRL and Regional Molecular Genetics Service, St Mary's Hospital, Manchester, United Kingdom. Molecular Genetics testing forms an increasingly important part of the diagnostic process in all branches of medicine. Studies of the reliability of such testing have indicated a significant level of inaccuracy in laboratory reports, arising from errors in sample identification, genotyping or interpretation. The European Molecular Genetics Quality Network (EMQN) aims to raise and maintain the quality of Diagnostic Clinical Molecular Genetics Testing by providing external quality assessment (EQA) schemes. In 2007 EMQN provided 18 disease specific and 2 technique specific EQA schemes. The EMQN's schemes are organised by a panel of experts and DNA samples are sent to participating laboratories once a year. Participating laboratories are asked to perform their routine analysis and interpret the results. The reports are marked by a group of experts. The participants receive a report on their performance.

400 laboratories from 42 countries around the world participated in the EQA schemes in 2007 and over 900 reports were evaluated from laboratories. The standards of genotyping accuracy were high but significant error rates were found and methods of reporting and interpreting data were varied. The error rate indicates a clear need for EQA to measure current standards of proficiency and encourage laboratories to raise their technical and reporting performance.

#### P09.45

##### Outcome of neonatal screening in Leningrad province in 2007

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Medical genetics service of Leningrad province is stationed at District Children Hospital being carried out in cooperation with the Department of Medical Genetics at MAPS and St. Petersburg Centre of Medical Genetics. Last year the genetic service was expanded with a neonatal screening for phenylketonuria, congenital hypothyroidism, congenital adrenal hyperplasia, galactosemia and cystic fibrosis. One infant with a high level of Phe and three infants with an increase in TSH were found among 10226 newborn babies. Three cases of congenital adrenal hyperplasia and none of cystic fibrosis risk were revealed among 4736 infants. Two babies (a girl and a boy) with galactosemia were found among 4942 infants screened. The girl had a GALT deficiency and presented a compound heterozygote Q188R/K285N; a molecular diagnostics was performed in Moscow Centre of Medical Genetics. Medical genetics service of Leningrad province comprises a confirmation of a hereditary diagnosis, medical care, long term inpatient and

outpatient care, dietary management and genetic counseling.

#### P09.46

##### Effectiveness of a program aimed to prevent Nonketotic Hyperglycinemia in a highly inbred community in Northern Israel

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**Introduction:** Nonketotic Hyperglycinemia (NKH) is an autosomal recessive metabolic disease characterized by the accumulation of large amounts of glycine in body fluids. Patients with NKH have severe neurological symptoms such as seizures. Most patients die within a few weeks, whereas survivors show severe psychomotor retardation and do not achieve any developmental milestone. NKH is caused by a mutation in the genes encoding the components of the glycine cleavage multi-enzyme system. More than 80% of the patients have defects in the gene encoding P-protein, whereas the rest of the patients have defects in the gene encoding T-protein. In recent years, prevention programs for the detection of heterozygotes of relatively prevalent autosomal recessive diseases (with a frequency of about 1:1000), is available in Israel. **Aim:** to study the carrier frequency of NKH in a village in Northern Israel where 20 patients, homozygous for the H42R mutation in the T-protein gene, were diagnosed over the last decade.

**Methods:** 405 healthy individuals in their reproductive period, residing in this village, were screened for the H42R mutation using PCR-RFLP method. **Results and conclusions:** A high carrier frequency of 1/16 (25 out of 405) was found. Since no effective treatment is available for NKH, prenatal diagnosis was requested in four pregnancies, which led to the diagnosis of three unaffected fetuses. One couple decided not to perform prenatal diagnosis. An affected baby was born and died at age 3 months. The availability of DNA testing enabled genetic screening and counseling in this small village.

#### P09.47

##### Genetic care of families affected by albinism in an African cultural context

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The birth of a baby with the autosomal recessive condition oculocutaneous albinism (OCA) in a black African population is often traumatic for the mother as the phenotype, with hypopigmentation of the hair, skin and eyes, differs so visibly from the normal level of pigmentation in this group. The incidence of OCA among the Venda of northern South Africa is relatively high, with 1 in 1970 affected, giving a carrier rate of 1 in 23. Myth and superstition surround OCA, with affected families believing they have been bewitched. The experiences of genetic nurses at the large regional hospitals in Venda and of families who have benefited from their genetic counselling services are explored via semi structured interviews. The genetic nurses, based in the midwifery section, identify affected babies as part of the National Birth Surveillance Programme and intervene immediately to inform the mother of the special health care needs of her baby. They explain the genetic causes of OCA and aim to empower her to counter negative attitudes she is likely to encounter from her family, who may accuse her of infidelity, and the wider community when returning to her rural home. Barriers to the provision of genetic care in this remote, rural region and strategies adopted by health care professionals to overcome these difficulties are explored. This genetic practice is a model for providing care to those affected by genetic conditions in rural, low resources regions of the world.

#### P09.48

##### Orphanet: What's in it for you? UK case studies

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Orphanet ([www.orpha.net](http://www.orpha.net)) is the largest international online resource for genetic and rare diseases. The recently improved website features a rare diseases listing and a directory of services alongside other specialised products which have been developed to better serve the rare disease community. This presentation uses case studies from the UK to highlight the information and services available to patients, health professionals and researchers.

Case study 1: Jane had a baby Jason who, at the age of 6 weeks, pre-

sented with nystagmus and photodysphoria. A few months later, Jason also suffered congestive heart failure due to dilated cardiomyopathy. Following visits to her local hospital, and a referral to Birmingham Children's Hospital, Jason was diagnosed with Alström syndrome. Jane had never heard of this condition before and uses Orphanet to find out more about it, and how to meet other parents with affected children.

Case study 2: Paul is a consultant clinical geneticist specialising in skeletal malformations. Paul has a patient with Schinzel Phocomelia and has learned, through the OrphaNews Europe newsletter, that a UK study is ongoing to discover genotype-phenotype correlations for this condition. He also uses Orphanet to find out that a laboratory in Berlin offers mutation analysis for the WNT7A gene, which causes Schinzel Phocomelia.

Case study 3: Samantha is a researcher who recently gained her PhD and then took a postdoctoral position working on Batten disease. Samantha uses Orphanet to discover what other research is ongoing for Batten disease and also to find contact details of potential collaborators.

#### P09.49

##### Protecting patient's confidentiality: opinions of russian medical geneticists

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The views of medical geneticists, working in Russian genetic consultations, on protecting the confidentiality of a patient and the availability of genetic information to his/her relatives, spouses and third parties were estimated. From 9 to 26 % of geneticists (depending on disease in question) considered it permitted to inform the relatives on the results of genetic testing without the consent of the patient. The significant part of geneticist (61.8%) considered, that samples of stored DNA, should be available to blood relatives of a patient without his consent. About a half of doctors considered, that the employer and the insurance companies could have access to the results of genetic testing with the consent of the patient, from 30 to 50 % - that such information can be given to schools with the consent of parents of the child. From 10 to 25 % of geneticists considered, that such information should be accessible to third parties without the consent of a patient. Thus, we could show, that Russian medical geneticists began to concern more respect to the right of a patient for confidentiality of genetic information then ten years ago.

#### P09.50

##### The role of Patient's Organizations in establishing some strategies in the healthcare system concerning rare diseases

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In Romania rare diseases are really orpheline. The project started by Romanian Prader Willi Association in 2007 (a project realized with the financial support of Trust for Civil Society in Central and Eastern Europe) induced multiple changes and it has a real chance that it's main objective to be accomplished. This objective is to develop the awareness of the community regarding patients affected by rare diseases by involving the social actors from this domain: patients, families and specialists. In the same project it was founded Romanian National Alliance for Rare Diseases (RONARD) whose objectives are: to organize informational campaigns concerning rare diseases, to adopt a national strategy in approaching rare diseases, lobby and advocacy activities to transform these strategies in a National Plan for Rare Diseases approved by the Ministry of Health. Today Romania is much closer to the European system in approaching patients with rare diseases with the support and guidance of EURORDIS (European Organization for Rare Diseases), Orphanet (a database with information about rare diseases and orphan drugs) and the involvement of the Ministry of Health, Labor Ministry and Ministry of Education. The activities sustained by RONARD (courses, conferences, media messages) in the relationship with authorities, specialists, patients and their families, but also the civil society in general, will guarantee the necessary environment to create a social and medical system in which Romanian patients with rare diseases will benefit from diagnosis, treatment and care conditions similarly as patients from other European countries.

#### P09.51

##### Policy harmonization and population biobanks

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Policymaking in an international context is often delegated to recognized international organizations (e.g. WHO, UNESCO, OECD). Similarly, international societies of concerned individuals and experts contribute to the elaboration of guidance, within the context of their cultural and national setting. Many of these groups have contributed policy recommendations that relate to the field of population genomics. But are the ethical, economic, environmental, legal and societal issues simply being multiplied and blurred by the sheer number of participants? At the Centre de recherche en droit public (CRDP) of the Université de Montréal, in Montréal, Canada, a Policymaking Core has been established to work with and inform the international community of the possibility and importance of prospective approaches to traditional issues such as consent, access, governance and commercialization, as they relate to population biobanks. If these issues are not discussed and some level of harmonization and clarity achieved, the population health goal of these ambitious projects will be hindered, due to the absence of policy interoperability. To this end, the Core collaborates with the Public Population Project in Genomics (P<sup>3</sup>G) member biobanks to create tools that will aid the harmonization of various aspects of biobank policy. Generic consent materials have been created and are publicly available from the P<sup>3</sup>G Observatory; future work will focus on governance and access issues.

#### P09.52

##### Burden of Genetic Disease in Colombia, 1996-2025

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Objective. To establish the load of genetic diseases in Colombia, according to population growth parameters until the year 2025. Methods. The frequency of new cases of genetic diseases and congenital malformations was calculated, establishing the potentially lost years of life, cause of incapacity and cause of death by these pathologies, weighing the data according to the growth and life expectancy of the Colombian population from 1996 until 2025. Results. Genetic diseases have a frequency that oscillates between 37.3 and 52.8 by each 1,000 inhabitants. Congenital malformation and birth defect frequencies are included, with a proportion of ≈ 50% of the studied genetic pathologies. Conclusions. There is a potential load of genetic disease that raises the need to implement more centers for the training of geneticists and auxiliary personnel who can in the future, provide suitable services, of diagnosis and genetic assessment.

#### P09.53

##### A retrospective analysis of clinical prenatal counselling activity performed by two clinical medical genetics centers in Emilia Romagna region, Italy (2000-2006)

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A retrospective analysis of clinical counselling records in two clinical genetics services in Emilia Romagna Region (Ferrara and Imola) is presented. A shared specific software (GCS) allows a systematic and homogenous collection of data. Diagnostic data were checked for correctness by medical geneticists. The study is focused on prenatal counselling in the period 2000-2006 (more than 9100 consultations) which represents >70% of the activity for both Medical Genetics Centres. The National Health System offers prenatal counselling for increased chromosomal anomalies linked to advanced maternal age ( $\geq 35$  years). These referrals represent 45% of prenatal counselling. In 2.9% of these prenatal settings, unexpected additional relevant reproductive risks have been discovered (1.7% mental retardation and 1.2% mendelian diseases) requiring an urgent and appropriate activation of clinical and laboratory diagnostic pathways. The referrals for the other prenatal consultations were heterogeneous mendelian diseases (29.2%) followed by chromosomal conditions (16.6%). Different proportions were seen in the two medical genetics services, reflecting the specialised activities as regional hub centres: hemoglobinopathies in Ferrara and cytogenetics in Imola. These specialised centres attract

women from other health districts for prenatal referrals, (40.7% from outside the provinces and 19.5% from outside the Emilia Romagna Region).

Data of the primary care and specialists access to genetic services, accuracy of prenatal diagnosis and time to diagnosis were analyzed to provide information for the analysis of the commitment, appropriateness of referrals and to improve the organization of clinical genetics services in the Emilia Romagna region.

#### P09.54

##### **Knowledge and understanding of prenatal screening and testing before and after the introduction of information booklet**

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Pregnant women considering prenatal screening need to have access to accurate information, facilitating informed choice. Uniform information about prenatal screening and testing was not available in Iceland prior to this study.

The study aim was to assess general knowledge about prenatal screening and diagnosis among pregnant women attending their first antenatal visit. An information booklet was produced as a part of this study. We compared the difference in knowledge and understanding between pregnant women in the intervention group (having access to the information booklet and normal care) (n=142) and women in the control group (normal care) (n= 237).

Women were recruited from five antenatal clinics in Iceland. The age distribution, experience and education of participants were in accordance with the general population. Overall 63% wanted information from their midwives and 31.7% from a doctor. Most had however, received information from their gynaecologist (53%) and friends (40%). The majority (60%) wanted both verbal and written information. The intervention group showed better knowledge when asked to explain the tests in their own words ( $p<.001$ ), but no significant difference was detected between groups in description of methods used in prenatal screening in multiple choice questions. When asked about specific disorders (Trisomies 13 and 18) women in the intervention group were more knowledgeable ( $p=.001$ ). We conclude that Icelandic women have a good basic knowledge of prenatal screening but want more information. There is a need for a better education on specific aspects of prenatal screening and diagnosis for women attending their first antenatal visit.

#### P09.55

##### **Development of molecular biology laboratory training courses for health care professionals at Nowgen, a centre for genetics in healthcare**

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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 research and clinical scientists in Central Manchester and Manchester Children's University Hospitals NHS Trust and the University of Manchester, has developed a portfolio of training courses for health professionals in response to expressed needs for training in practical molecular genetics laboratory techniques.

The first of these training courses - a national, two-day course for clinical cytogeneticists, accredited by The Royal College of Pathologists - was delivered in January 2007, with good training outcomes. Owing to high demand, the course was run a further three times in 2007 and 2008, reaching over 80 clinical cytogeneticists to date. Further training

courses have been developed and run with good outcomes, including a course tailored to the training needs of genetic counsellors and nurses. New courses have been launched in 2008, covering bioinformatics, quantitative & real-time PCR and biobanking.

All courses have been developed following initial consultation with the relevant professional groups' senior representatives. Following this, to determine specific training needs, questionnaires are sent to heads of individual units, asking about the optimum length of courses and which key laboratory techniques should be included.

#### P09.56

##### **Genetic Counselling in pulmonary arterial hypertension: experience from the French Reference Centre**

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Pulmonary Arterial Hypertension (PAH) is a rare condition (prevalence from 15 to 50 cases per million) characterized by an increase in pulmonary vascular resistance leading to right-heart failure and death. This condition has a poor prognosis, although novel treatment options allow better quality of life and survival. Recent data indicate that early diagnosis and treatment should translate into better outcome and survival. PAH can be sporadic, associated with other conditions such as systemic sclerosis, or clustered in families.

Familial PAH currently represents at least 10% of so-called idiopathic cases. It segregates as an autosomal dominant trait with reduced penetrance (10-20%). Mutations of the gene encoding bone morphogenetic protein receptor type II (*BMPR2*) which belongs to the TGF  $\beta$  super family have been first described in 2000. 20% of so-called sporadic idiopathic cases and more than 70% of familial cases are characterised by germline *BMPR2* mutations, emphasising the relevance of genetic counselling in these families, in order to promote early diagnosis and treatment in at-risk subjects. We thus set up a genetic counselling clinic in our department. In the last 5 years, we screened 375 PAH patients (310 sporadic, 65 familial), and 125 first-degree relatives of patients harbouring a *BMPR2* germline mutation. 45 sporadic, 37 familial and 45 first-degree relatives have been shown to harbour a *BMPR2* mutation. First-degree relatives carriers received detailed information on the characteristic symptoms of the disease (mostly dyspnea, chest pain and syncope) and screening tools such as Doppler echocardiography (which should be repeated every year).

#### P09.57

##### **Quality management and accreditation of genetic testing services**

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Governments, regulators and professional bodies have a responsibility to ensure that all genetic testing services are offered within a quality assurance framework that retains the confidence of the public. The recently published OECD guidelines for Quality Assurance in Molecular Genetic Testing are equally applicable to Cytogenetics & Biochemical Genetics. Agreement on appropriate standards in genetics, and how to measure them, will assist in harmonizing the quality of service across the European Union. External Quality Assessment (EQA) is an important instrument to reach the harmonization and quality improvement aimed for by the EuroGentest Network. The introduction of reference materials into the diagnostic service and the validation of new technology, as it moves from research to the diagnostic arena, are essential and agreement on best practice for standards and strategies can be achieved by consensus. In addition, Best Practice Guidelines for Cytogenetics (ECA), Molecular Genetics and Biochemical Genetics have been supported through the EuroGentest Network and published.

EQA and accreditation are recognized by international standards (ISO)

bodies as a tangible measure of the quality of a laboratory's performance as it provides a consistent standard to which services should aspire. Accreditation of laboratories will be valuable in the harmonization of standards and strategies, in the assessment of emerging technologies, as well as in the provision of training and education.

The Quality Management Unit of the EuroGentest Network is involved improving the quality of diagnostic services in all these areas, through training, education, reference materials, new EQAs, best practice guidelines, SOPs and validation documents.

#### P09.58

#### Quality and risk management for molecular genetic testing in the laboratory and clinical settings: A perspective from the United States

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A Quality / Risk Management Model is proposed for the provision of molecular genetic testing services. This Model emphasizes critical junctures in the testing process where the assembly and communication of clinically relevant information is necessary for appropriate patient management. The evidence-base for this model is derived from earlier studies and particularly those addressing the provision of DNA-based testing for cystic fibrosis in the United States. The Model emphasizes the pre- and post-analytic components of the testing process. Critical components include, among others, initial decision to order the test, efforts taken to collect and communicate patient/family information prior to testing (i.e., relevant clinical information, family history, race/ethnicity), specimen type, handling, and transport, laboratory evaluation of the test request, specimen adequacy, selection of methodology, development of the test report, communication of the result to the clinical setting, and evaluation of the report toward informing appropriate clinical decision making. The role of personnel competency, integration of professional guidelines into practice, costs, and variation in practice settings are commented upon. It is envisioned that such a Model may be useful as a tool for identifying and developing interventions to reduce risk and improve performance to promote high quality testing services in the United States and may have elements applicable elsewhere.

#### P09.59

#### The role of volunteers in activities supporting Rare Diseases Alliance

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The problematic of Rare Diseases in Romania was a true Cinderella until 2000. In the latest years due to the initiative of NGO is this pathology caught the attention of medical people by the means of the model that was offered in Europe, where the role of volunteers derived from families, universities and NGO is become essential.

In the activity of NGO Save the Children, Timis branch in partnership with pediatricians and the University of Medicine and Pharmacy Timisoara was initially penciled with two Associations of Patients suffering of genetic diseases (Down syndrome and Prader Willi Syndrome). Using the young group of volunteers that have worked with patients suffering of Down syndrome and using the expertise of Genetic Chair, was initiated collaboration by many specialists and NGOs resulting the National Alliance of Rare Diseases in Romania. Now we have more than 15 Associations I the Alliance.

The Volunteers of Save the Children, Timis branch participated at the first National Conference of Rare Diseases (2007 Zalau, Romania). In collaboration with specialists we have trained a number of 20 young volunteers from Save the Children Timis. Now they are preparing the activities for a week dedicated for Rare Diseases in each February (mass media campaign, street campaign about this theme, round table with specialists, patients, volunteers and family patients).

#### Conclusions

The role of young volunteers within this Association was essential. The volunteers of Prader Willi Romanian Association and Save the Children Timis have coordinated the first activities for those pathologies patients.

#### P09.60

#### Genetic counseling in familial autosomal reciprocal translocations

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Reciprocal translocations are common chromosomal rearrangements resulting in an increased risk of a segmental aneuploidy in children of a carrier. The reciprocal translocation carrier families are interested in their risks of having an unbalanced offspring. Segregation analyses of pedigrees are important in such families before genetic counselling is given. In the current study, we examined the three generation pedigree analyses of 17 cases with autosomal reciprocal translocations with different indications for chromosome analyses. Karyotype analyses of Giemsa-trypsin banded chromosomes were made after the first genetic counselling session during which a three generation pedigree was drawn. Of all the patients, 7 cases were consulted because of recurrent abortuses, 3 cases of infertility. 1 case had a previous child with congenital abnormalities and 1 case had a known familial reciprocal translocation. 5 cases were detected prenatally. Of these, 2 had increased Down syndrome risk in an ongoing pregnancy. Amniocentesis was performed in 2 cases because of advanced maternal age and in 1 case because of a known reciprocal translocation in mother. We detected reciprocal translocation in all cases. During the second genetic counselling session, the patients were informed about their magnitude of calculated risks of having an abnormal child, about the possible abnormalities of the offspring and risks of pregnancy losses. As for the infertile couples, the possibility of preimplantation genetic diagnosis was also discussed as well as the impact of the translocation on gametogenesis.

#### P09.61

#### Certified Reference Materials for genetic testing

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The lack of tailored reference materials (RMs) and reference measurement procedures for genetic testing slow down the validation and development of new assays and question the reliability of results as tests might potentially be performed in absence of adequate quality controls. The IRMM, as accredited reference material producer (ISO Guide 34), has released in 2006 a set of three certified RMs to be used for the detection of the G20210A mutation in the human prothrombin gene. The materials consist of a 609 bp fragment of the gene (wildtype or mutant sequence) inserted into a pUC18 plasmid. The certification procedure, including the methods used, is described in detail in the certification report and discussed in a recent publication (Clin Chem Lab Med, in press). These activities support the efforts of the genetic community to improve the quality assurance and the harmonisation of genetic testing in Europe and beyond. In addition, IRMM has issued, in the frame of the European Network of Excellence EuroGentest, a guidance document on the use of RMs in genetic testing. This document tackles, with internationally recognised definitions, the state-of-the-art in the field, the selection criteria for RMs according to their use and provides examples for better understanding of the requirements in the field. This presentation will provide an overview on both developments and an outlook to further needs.

#### P09.62

#### The Genetic Testing Reference Materials Coordination Program (GeT-RM)- A sustainable community process to improve availability of appropriate reference materials for genetic testing

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**BACKGROUND:** The expansion of clinical molecular genetic testing has increased the need for appropriate and characterized cell line/genomic DNA reference materials for quality control, test validation, proficiency testing, and development of new genetic tests. However, despite the growing test volume and the rapidly increasing number of tests offered, the necessary reference materials are not available for the vast majority of tests. **METHOD:** The U.S. Centers for Disease Control and Prevention (CDC), in collaboration with the genetic testing

community, has established the Genetic Testing Reference Materials Coordination Program (GeT-RM) to improve public availability of reference materials and facilitate information exchange and communication on reference materials development, contribution, characterization, distribution, and needs assessment. RESULTS: The GeT-RM has characterized reference materials for Huntington disease, Fragile X, cystic fibrosis, Bloom syndrome, familial dysautonomia, Canavan disease, Niemann-Pick disease, Tay-Sachs disease, Gaucher disease, glycogen storage disease type 1a, Fanconi anemia, and mucolipidoses type IV. The GeT-RM program also collects information from other sources about publicly available cell lines/DNA with clinically important mutations that may be useful as reference materials. This information is posted on the program website. To date, the GeT-RM has focused its efforts on DNA based testing for inherited genetic disorders. However, we hope to expand our efforts into other areas of genetics, including molecular oncology and biochemical genetic testing. CONCLUSIONS: The increased availability of characterized reference materials for quality assurance, proficiency testing, test development and research, will help to improve the quality and accuracy of genetic testing. GeT-RM website: <http://www.cdc.gov/dls/genetics/rmmaterials/default.aspx>

#### P09.63

##### The role of AZFc region microdeletion in repeated pregnancy loss

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Objective: To evaluate the role of Y chromosome microdeletion in the case of couples had problem with repeated pregnancy loss (RPL) in-group of unknown cause compared with couples with male factor infertility.

Materials & Methods: A controlled clinical study was designed in our centre. In total, 100 men from male factor infertility and 25 men with RPL were recruited in the study.

DNA was extracted from Peripheral blood sample. In each sample, six sequence-tagged-sites (STS) according to the European protocol and four other STSs in the proximal AZFc region namely; DYS262, DYS220, DYF8551, and DYF8651 were studied by polymerase chain reaction (PCR).

Results: Eight men tested evaluated had Y microdeletion in at least one of the six segments from European protocol (8%) VS non in RPL group. Five men with history of at least 3 PL in their wife tested for 4 STS in AZFc region had microdeletion (20%) but none of those other STS. In the infertile group had not any microdeletion related to the 4 STS from AZFc region.

Conclusion: From our results it could concluded that in case of couples with un explained PRL it may help to test these four STSs on Y chromosome to recognize the cause of PL.

#### P09.64

##### Mutational homogeneity of severe autosomal recessive retinal degeneration in endogamous Romany (Gypsy) communities in Slovakia

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Hereditary retinal degenerations comprise clinically and genetically wide group of mostly progressive ocular disorders and are one of the most frequent causes of vision impairment in developed countries. So far uncovered tremendous genetic heterogeneity underlying majority of these disorders however markedly impedes ongoing attempts on DNA diagnostics and therapy in most of populations. Contrary to panmictic populations, genetic heterogeneity in isolated populations is often reduced to high degree due to founder effect and inbreeding. Severe childhood autosomal recessive rod-cone progressive retinal degeneration with feature of retinitis pigmentosa (nyctalopia, dark pigment formations, attenuated retinal vessels) and nystagmus characteristic for LCA phenotype occurs in higher frequency in to the high degree isolated Romany communities in Slovakia. Whole genome autozygosity mapping and linkage analysis accomplished on analysis of two large pedigrees facilitated localization and identification of underlying mutation to which bidirectional allele specific amplification method

was designed for routine mutation detection. Geographically delimited occurrence of the disease confirmed community structure of Romany population with existence of small, endogamic founder settlements. Growing of such small communities, characterized by high consanguinity rate, results in markedly increased incidence of this autosomal recessive disorder in some regions leading to significant genetic burden to the population. While no effective therapy exists for the disorder at the moment, cascade carrier screening and genetic counseling proffered to the families is currently the only possible approach to the management of the disease.

#### P09.65

##### Governing the balance between 'duty to protect' and 'right to test' II: New forms of protection in genetic screening

**M. C. Cornel, T. Pieters, C. G. van El;**

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While in the previous century the availability of genetic testing and screening was governed by public health authorities and medical professionals, increasingly direct-to-consumer tests become available. While on the one hand people have become more free to decide for themselves whether they want to order their genetic risk test, on the other hand, lack of counselling, personalized advice resembling general lifestyle advice or incorrect results may lead to disappointment or may prove harmful. Quality of genetic services is no longer guaranteed by existing regulations.

In the United Kingdom the Human Genetics Commission has updated its recommendations concerning direct-to-consumer testing, calling for stricter control, without precluding commercial testing, for instance by recommending that the test be offered by a qualified health professional. To guarantee informed decision making, the public needs to have access to high quality information (trusted websites). Education and communication are needed to inform the public at large on pros and cons of genetic screening. Policy makers should engage with patient and consumer organisations. Especially for complex diseases translational research is needed. Possibilities to regulate marketing of commercial test offers after adequate pre-market evaluation need to be debated and implemented. The European certificate CE marking should guarantee analytical validity and clinical validity. Assessment of clinical utility, relating to the availability of effective interventions, should be performed for new forms of testing and screening for low-risk populations and high as well as low-risk gene variants.

#### P09.66

##### Governing the balance between 'duty to protect' and 'right to test' I: Challenges to the focus on protection in genetic screening

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For genetic screening, criteria have been developed based on the WHO Wilson and Jungner criteria (1968), in which the notion of protection of citizens against unwarranted screening was important. In some countries, such as the Netherlands, 'protection' even became cornerstone of legislation concerning population screening. Protection relates to notions of having a suitable test with good sensitivity and few false positives, the availability of treatment and follow-up after diagnosis, so that benefits of screening outweigh potential harm, for instance of stress caused by uncertainty, the knowledge of having a fatal disorder or unnecessary interventions.

In screening policy safeguards are needed because of the shared responsibilities of government and health care taking the initiative to offer screening to - in principle - healthy people.

We argue that recent developments challenge this protective stance. Firstly, on the basis of developments in genomics, among other association studies, labs and companies increasingly offer direct-to-consumer tests on the internet. Secondly, there is increasing awareness about and demand for testing and screening, sometimes stimulated by patient organisations. For instance, in the Netherlands, parents of children with Duchenne Muscular Dystrophy argue that people might want a newborn screening test for DMD to be able to prepare or make reproductive decisions.

The emphasis on protection ignores increasing demands for testing. Citizens want to be more autonomous. "Informed decision making" is not taken seriously when many genetic screening tests are simply not

made available. New ways to involve the public in policy decisions on screening and testing are necessary.

#### P09.67

#### The importance of genetic counselling for parents with normal constitutional karyotypes having multiple spontaneous abortions with aneuploidy

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The purpose of this research is to establish the hypothesis that two hits are responsible for aneuploidy which results in spontaneous abortion. The first hit could have been triggered by patient's former diseases of urinary and/or genital system. The number of spontaneous abortions recorded in their siblings of second generations creates predisposition that may end with termination of pregnancy. The second hit is usually triggered by chronic presence of EBV virus in male or/and female partner. It has been demonstrated that there is no significant relation between the age of male or female partners and the number of spontaneous abortions. Both males and females had former urinary or/and genital infections and the number of spontaneous abortions among the siblings of second generation is twice as high in relation to average population. Variations in constitutional karyotypes (per inv 9, 9qh+) are also evident as well as presence of antibodies for EBV, CMV, or HSV2 viruses. Reactivation of latent EBV infection provides possibility for second hit. Aneuploidy or tetraploidy is found at 23% of samples. Therefore we think that the presumed hypothesis of two hits is clearly established. Partners with spontaneous abortion with and without chromosomal abnormalities must undergo genetic counseling process for preventing trigger/s or to repeat the process, because spontaneous abortion is a multifactorial disease.

#### P09.68

#### Milestone in teaching genetics

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On the verge of a new legislation in the educational system, when one of the main goals is the alignment to the international standards, it is suggested that student's evaluation has to become mandatory and its results should be made public by the university. More than a decade ago I evaluated my teaching methods and presented the results at the ESHG meetings. Three years ago I decided to change the way I taught the practicals.

Questionnaires were answered by 76 first year students of the Medical and Pharmacy University "Carol Davila" to evaluate the quality of the practicals they attended during the first term in the Department of Genetics. They were questioned about the quantity of information versus amount of time, the quality of information and presentation and if they enjoyed the practicals.

Answers to the question: Have you been satisfied with the way the practicals unfolded?

very much	7%
a lot	42%
enough	50%
satisfactory	1%
almost not at all	none
not at all	none

For several years I have stopped asking students for an evaluation, because the results were the same: almost half in favor of frontal teaching and the other half preferring the interactive method. Since I have modified my teaching method, the speed and the amount of information also changed, but only 7% of students considered it was too much data.

Adapting the teaching method to the students' present needs makes them more prone to enjoy the practicals. The study did not focus on achieving genetic knowledge, but apparently this did not improve.

#### P09.69

#### Thrombophilic gene mutations: two different approaches to the validation of commercial and "in-house" DNA diagnostic assays

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Validation of molecular genetic analytical methods is the basic tool for quality assurance and has to be part of routine practice in clinical-diag-

nostic laboratories that are working in compliance with the ISO15189 guidelines ([www.iso.org](http://www.iso.org)). Moreover, OECD has issued "Guidelines for Quality Assurance in Molecular Genetic Testing ([www.oecd.org/sti/biotechnology](http://www.oecd.org/sti/biotechnology))" which stipulate that laboratories providing clinical services should be working within a "Quality Assurance System". Via method validation laboratories assess assay performance and demonstrate their suitability for the intended purpose. It is up to individual laboratories to establish an acceptable validation protocol in order to comply with general ISO principles. Here we report practical examples of validation procedures utilized for an „in-house“ test that we developed for the detection of thrombophilic mutation PAI-1, versus approaches applied to a commercially produced RHA Kit Thrombo (Labo Bio:Medical Products; CE/IVD marked). For examination of the thrombophilic mutation PAI-1 our laboratory uses an "in-house" method based on fragment analysis of restriction endonuclease-digested amplicons, while the commercial assay is based on reverse slot hybridization (RHA) for simultaneous detection and identification of mutations in the genes coding for FII G20210A, FV-Leiden, including the MTHFR 677C/T variant. In both instances we assessed basic validation parameters including specificity, sensitivity, repeatability, reproducibility and robustness. Differences between both validation protocols are within the scope of validation parameters being tested. These procedures enabled us to confirm diagnostic reliability of both techniques and provide practical examples for other laboratories validating their genetic tests. Supported by VZNM00064203(6112) and EuroGentest.

#### P09.70

#### Genotyping of MTHFR 677C>T and 1298A>C variants by high resolution melting of small amplicons: an example of method validation.

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High Resolution Melting (HRM) is a simple, cost-effective and rapid mutation scanning method. By reducing the respective amplicon size, it is possible to increase the resolution for reliable discrimination between homozygous wild-type and homozygous mutant samples. We introduced HRM close-tube method in our laboratory for genotyping of the most common variants in the MTHFR gene: 677C>T (rs1801133: C>T) and 1298A>C (rs1801131: A>C). PCR amplification and HRM was performed on the LightCycler® 480 Real-Time PCR System (Roche). For validation purposes we evaluated specificity, sensitivity, repeatability, reproducibility and the ability of the method to perform reliable calls. We were able to perform unambiguous calls in 98.4% (n=381) of the cases for rs1801133: C>T and in 98.1% (n=104) of the cases for rs1801131: A>C. For the 6 (1.6%) and 2 (1.9%) unknown samples for rs1801133: C>T and rs1801131: A>C respectively, by repeating the analysis we reached 100% of calls. Since in the end all calls were reliably detected by the LightCycler software we reached 100% specificity and sensitivity. Repeatability and reproducibility were also consistent. In conclusion, after optimization and diagnostic validation HRM of small amplicons is a reliable and precise method that can be broadly applied in DNA diagnostics of other genes.

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#### P09.71

#### Training on quality management to support genetic testing laboratories in continuous improvement and progress towards accreditation

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ISO 15189:2007, the international standard developed specifically for medical laboratories, requires that staff receives relevant training in quality assurance and quality management (5.6.1). In response to the growing interest in accreditation and quality improvement, a series of interactive two-day training workshops were organized for genetics laboratories, within the framework of the European project EuroGentest.

The subjects of these workshops varied from living with quality systems and comparing the different standards for accreditation, to specific topics such as internal audit, managing the human side of change

processes and IT support for quality management.

Since 2005, 156 different participants, from 85 institutes and 27 countries worldwide, have participated in the 8 workshops organized to date.

In addition to the training sessions, an electronic survey was sent to all participating institutes in order to get a more detailed view on the progress laboratories make towards accreditation and the implementation of different quality aspects such as document control, performing internal audits and tracking non-conformities. Furthermore, the value of training in the improvement of a quality system was surveyed. The answers received from more than 50 institutes have been analysed. This survey gives us insight into the reaction of laboratories to the increasing pressure to develop their investment in continuous improvement and accreditation. Moreover, we will anticipate and address topics on quality in future workshops, particularly adapted to the needs of the genetic testing laboratories.

#### P09.72

##### **Genetic consulting of families when one of the members is the carrier of balanced translocation**

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Infertility in the family is one of most common courses of genetic consulting. Between 266 consulted families we found 4 families with carrier of balanced translocation. By the example of these families we'll try show the specific features of their genealogies and ethical aspects of genetic consulting. Translocation examples in the families:

1. Male/Female: 46 XY, t (1;4) (p36.2; q32)/ 46 XX.
2. Male/Female: 46 XY/ 46 XX, t (4;9) (p15.3; q12).
3. Male/Female: 45 XY; -13; -14; +t (13;14)/ 46 XX.
4. Male/Female: 46 XY/ 46 XX, t (6;10) (q23; p13).

Genealogies of these families had some characteristic features:

1. Sterility.
2. Spontaneous abortions and miscarriages.
3. Stillborns.
4. Different anomalies of fetus.
5. The cases of mental retardation of unclear etiology between relatives.

Some ethical problems rise during genetic counseling of these families:

1. Who must say the results of chromosomal examination for healthy spouse - doctor or translocation suffering husband or wife?
2. What tactic should keep the doctor in cases when translocation carriers don't agree inform other member of family?
3. The examination of first line relatives (parents, brothers, sisters and children) and other relatives sometimes needs to avoid more fetal anomalies. What tactic must be of the doctor in case when couple doesn't want informing others? Is it ethical to hide information which knows the doctor from relatives who might be interested in?
4. How explain family the results of examination in correct way to avoid conflicts and sometimes divorces of the families?

#### P09.73

##### **Towards practical guidelines for the validation of genetic diagnostic tests**

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Continuous advances in human genetics and biotechnological developments have led to a rapid growth of nucleic acid-based diagnostic testing in medical laboratories. To further improve the quality of genetic testing, it is necessary to support the implementation of reliable tests.

Several quality assurance measures are demanded by regulatory organizations and accreditation bodies as outlined in published standards and guidelines (e.g. ISO 15189 standard, the IVD Directive 98/79/EC, the OECD Quality Assurance Guideline).

However, proper instructions or the know-how to fulfil these requirements, especially for the validation of genetic tests, are lacking.

A working group was created within EuroGentest, that aims to develop practical guidelines for - to begin with - the analytical validation of molecular genetic tests.

Through brainstorming meetings in Leuven (2007) and Prague (2008), literature studies and with the help of external experts, a background document was generated. It includes a consensus glossary of terms (applicable to molecular genetic testing), a categorization of the variable array of genetic test methods, discussions on the meaning of critical performance characteristics for genetic tests and several generic aids, such as a flowchart to guide the user to appropriate instructions, a generic validation SOP and a template file for the validation report. Future plans include the experimental design of test validations, a statistical evaluation of 'numbers' in validation experiments, interlaboratory (collaborative) trials and clinical validation.

The document will be posted and submitted to a public and expert consultation during the summer of 2008.

#### P10. Therapy for genetic disease

##### P10.01

##### **Comparative sequence analysis, structure prediction, primer design of ApolipoproteinE protein in Alzheimer's disease**

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Alzheimer's disease (AD), neurodegenerative disease, is common cause of dementia, characterized clinically by progressive intellectual deterioration together with declining activities of daily living and neuropsychiatric symptoms or behavioral changes. Researchers have identified increased risk of developing late-onset AD to the apolipoprotein E (apoE) gene found on chromosome 19. In this study we have compared the apoE gene and its protein in different organisms to understand the mechanism of expression of apoE. Multiple sequence alignment (MSA) of the apoE gene revealed that it is analogous to all the other 16 organisms whereas the apoE protein is found to be 66% homologous. On analysis of apoE (NM\_000041), it was found that ORF region was between 84-1034 position. Specific primers for the apoE coding region were designed which resulted in product size 1109 bp. The apoE protein analysis showed that it was hydrophilic. 13 antigenic determinants were found when the apoE protein was analysed for Antigenic prediction sites which could be used as potential targets. The positions of alpha helix and b-sheets in the secondary structure of the proteins were predicted along with 3D structure prediction of apoE. The degenerate primers designed could be used as a diagnostic tool for identifying apoE protein expression in AD patients. The predicted antigenic sites on the apoE proteins could be used as an effective target for interaction with candidate drugs for the control of the expression of the apoE in AD patients, this has to be further evaluated using in vitro and in vivo methods.

##### P10.02

##### **Anthracyclines, an antibiotic family with anti-proliferating activity, can elevate $\gamma$ -globin expression *in vitro* and increase HbF production in human erythroid cells**

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Anthracyclines are a family of natural products produced by microorganisms of the actinomycetes group. Some members of the anthracycline group of antibiotics show pharmacological and mostly anticancer activities. Their biological activities are proposed to be the result of drug-induced damage or structural alterations to DNA due to inhibition of enzymes that are involved in the DNA synthesis like topoisomerase II.

In this study we investigate whether some anthracyclines could be HbF inducers due to their DNA binding properties and the formation of stable complexes with it. A group of other DNA binding agents were

found to be strong HbF inducers. This group includes compounds like cisplatin analogs, Chromomycin-Mithramycin, Tallimustine and An-gelincin. Some Ribonucleotide reductase inhibitors like Hydroxyurea, Didox and Trimodox were also found to be strong HbF inducers. Ribonucleotide reductase is an enzyme that plays an important role to DNA replication and repair. These properties therefore amplify our speculation that Anthracyclines might be inducers of HbF. We examined the following compounds: Doxorubicin, Aclarubicin, Idarubicin, Bleomycin and Daunorubicin on  $\gamma$ - and  $\beta$ -globin gene promoter activity in a dual luciferase assay in the GM979 cells. All of them increased the  $\gamma$ -globin promoter activity over 2 fold and were then tested in erythroid liquid cultures derived from normal donors. Doxorubicin, Idarubicin and Daunorubicin showed increasing levels of HbF and were then tested to thalassemic patients (preliminary results). The findings so far from all the measurements clearly show that the family of Anthracyclines could be a possible target for finding new HbF inducers.

#### P10.03

##### Medical rehabilitation for life quality improvement in a case with arthrogryposis

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Arthrogryposis is a congenital disorder characterized by multiple joint contractures found throughout the body at birth. We present the case of a girl of 3.5 year-old with multiple congenital defects: arthrogryposis involving bilateral hip, knee and ankle joints, together with sacral agenesis with lumbar dysmorphism, anorectal agenesis, hydronephrosis of the left kidney as the result from reflux, right kidney hypoplasia, renal fusion and heart anomalies: tetralogy of Fallot. Immediately after birth, in several steps, colostomy, left ureterocystostomy and suprapubic cystostomy were performed. Later in infancy corrective surgery for the heart defect was required. At the age of 2 years, surgery for the equinovarus deformities and for left genu flexum was performed. Medical rehabilitation tries to maximize independent function. The main goals were increasing the muscle tonus of upper-limbs, increase the rate of motion of the joints, establishment of stability for ambulation, learning different schemes of movement according to her needs, obtaining of a functional independence. The patient followed a complex rehabilitation program with hydrokinetotherapy, electrotherapy, massage, occupational therapy, psychological counseling. The therapies were successful, after 10 weeks an improvement of the moving capacity and of the transfer in orthostatism with minimal external assistance, with the obvious increasing of the patient's satisfaction, were noticed. In order to fulfill our objectives the child will be hospitalized every two months for functional evaluation and for continuing the physical therapies.

#### P10.04

##### Delineation of the 5-Azacytidine pathway by use of siRNAs

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Induction of  $\gamma$ -globin may become a useful means for treatment of  $\beta$ -thalassemia. 5-Azacytidine (5-Aza) is a chemical compound that leads to the induction of  $\gamma$ -globin, at least in part through blocking the function of methyl transferases. In addition, it is known that the Methyl Domain Binding Protein 2 (MBD2) binds to methylated DNA and may be involved in repressing  $\gamma$ -globin expression. Therefore the function of 5-Aza may in part be mediated through blocking MBD2 binding to DNA. Here we have looked at whether the K562 erythroleukemia cell line is an appropriate model system for studying this pathway. We have used non-radioactive northern blot analysis and real time PCR to show that  $\gamma$ -globin can be induced in K562 cells. In addition, we have validated the function of a published MBD2 siRNA sequence by showing that it can knock down MBD2-specific mRNAs in HeLa cells. We are in the process of testing these siRNAs in K562 cells for 1) MBD2 knockdown and 2)  $\gamma$ -globin induction. Upon validation, K562 cells will be used further to delineate this pathway.

#### P10.05

##### Studying the $\gamma$ -globin induction pathway by hydroxyurea in K562 cell lines

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$\beta$ -thalassemia is a genetic disorder which may be ameliorated by induction of the fetal  $\gamma$ -globin gene. Several chemical agents can lead to the induction of  $\gamma$ -globin. One such chemical is hydroxyurea. This chemical is the only drug approved by the Federal Drug Administration (FDA) for treatment of  $\beta$ -thalassemia. Despite its use in  $\beta$ -thalassemia treatment, the mode of action hydroxyurea is poorly understood. Here, we have examined the effect of different hydroxyurea concentrations (50, 100, 200  $\mu$ M) on  $\gamma$ -globin induction in the K562 erythroleukemia cell line. We have used non-radioactive Northern blot analysis and RT-Real time PCR to measure  $\gamma$ -globin levels. We have determined that there is a 3-4 fold induction of  $\gamma$ -globin using 100  $\mu$ M and 200  $\mu$ M hydroxyurea concentrations. We have also optimized transfection conditions of K562 cells to show the effect of positive and negative control siRNAs. We are currently in the process of testing siRNAs against candidate gene(s) involved in this pathway.

#### P10.06

##### Congenital Disorder of Glycosylation type Ia: antisense therapeutics for an intronic variation causing exonization of an intronic sequence

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Congenital disorders of glycosylation (CDG) are a group of genetic diseases resulting from defects in N-glycosylation of glycoconjugates. The most common form of the disease is the CDG-Ia caused by a deficiency in the cytosolic enzyme phosphomannomutase (PMM). PMM converts mannose-6-phosphate to mannose 1-phosphate and is a key enzyme in the generation of N-linked glycans. The clinical presentation of PMM deficiency ranges from very severe to milder phenotype. In this work we report the functional analysis of a nucleotide change identified in the deep intronic region of intron 7 (c.639-15479C>T) of PMM2 gene and the use of antisense morpholino oligonucleotides (AMOs) to restore normal splicing. First, we have confirmed using splicing assay that this change provokes the exonization of 123bp between exon 7 and 8 in patient's cDNA. Using modified morpholinos to block access of the spliceosoma to 3' and/or 5' cryptic splice site we have demonstrated that this insertion is a disease-causing mutation. After transfection of a fibroblast cell line from the patient, the analysis of the effect of the AMO was done by conventional RT-PCR, determination of PMM activity and western blot using polyclonal antibodies. The results obtained shown that a correctly spliced mRNA was rescued and efficiently translated in a functional protein detected by western blot. PMM activity was rescued close to the value of control cell line. Our results offer a novel promise mutation-specific therapeutic approach in this genetic disease where is not possible other effective treatment.

#### P10.07

##### Treating congenital defects: a proposal of algorithm for nutritional intervention in individuals with cleft lip/palate

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Cleft lip and palate (CL/P) is one of the most common birth congenital defects, occurring in approximately 1/600- 1000 newborns babies worldwide. In view of the feeding difficulties presented by children with a cleft lip and/or palate and the importance of food in their growth and development, they should receive early and systematic healthcare, by a specialized team besides of regular pediatric care. Therefore, the search for strategies of low-cost and good effectiveness and improve of health care are recommended by the World Health Organization (WHO).

The diet of a child with a CL/P is critical for growth and development besides adequate gain in weight, which is important to corrective surgery at the right time. Despite of the high prevalence and the importance of nutritional intake, there are around 50 articles involving nutritional approaches over 50 years. We present a proposal of an algorithm for multiprofessional intervention for nutrition of CL/P babies before surgery.

#### P10.08

##### Mechanism of CMT1A phenotypic correction by high dose of ascorbic acid

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Charcot-Marie-Tooth [CMT] syndrome is the most common hereditary peripheral neuropathy usually results from triploidy of the PMP22 gene. Preclinical trials using an animal model show that disabled mice force-fed with high doses of ascorbic acid partially recover muscular strength after a few months of treatment, and suggest that high doses of ascorbic acid repress PMP22 expression (Passage et al, Nature medicine, 2004).

PMP22 gene expression was under the control of cAMP stimulation (Saberman et al, Gene, 2000). Recently we demonstrated that ascorbic acid represses PMP22 gene expression by acting on intracellular cAMP levels and adenylate cyclase activity. This action is dose-dependent and specific to ascorbic acid, since repression is not observed after treatment with other antioxidants (Kaya et al, Neuromuscular Disord, 2007). Indeed we showed that ascorbic acid is a competitive inhibitor of cyclase adenylate enzyme. This work enabled us to propose an unsuspected mechanism of action explaining the phenotype correction, and to identify two new potential therapeutic targets: the ascorbic acid would act directly on the PMP22 gene expression by decreasing intracellular cAMP concentration via the inhibition of the adenylate cyclase activity.

#### P10.09

##### U1snRNA-mediated rescue of mRNA processing in severe factor VII deficiency

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Small nuclear U1-RNAs (snRNAs), the spliceosome components selectively recognizing donor splice sites (5'ss), were engineered to restore correct mRNA processing in a cellular model of severe coagulation factor VII (FVII) deficiency, caused by the IVS7 9726+5g/a change. Three U1-snRNAs, complementary to the mutated 5'ss (U1+5a) or to neighbouring sequences, were expressed with FVII minigenes in a hepatoma cell line.

The U1-snRNAs reduced from 80% to 40% the exon 7 skipping, thus increasing exon definition. The U1+5a construct also dramatically increased recognition of the correct 5'ss over the 37bp-downstream cryptic site preferentially activated by the mutation, thus inducing appreciable synthesis of normal transcripts (from barely detectable to 50%). This effect, which was dose-dependent, clearly demonstrated that impaired recognition by the U1-snRNA was the mechanism responsible for FVII deficiency. These findings suggest compensatory U1-snRNAs as therapeutic tools in coagulation factor deficiencies caused by mutations at 5'ss, a frequent cause of severe defects.

#### P10.10

##### Influence of proprioceptive training program in children with Down syndrome

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**Background:** In these days, Down syndrome comes again in the concern of medical researchers because early intervention treatment can improve the quality of life of these children. The goal of our study was to prove the efficiency of a proprioceptive training program on the motor development stages of children with Down syndrome.

**Methods:** The study was performed between September 2006 - September 2007. The study was performed on 30 children from the "Speranta" Special Care Center Timisoara (16 girls and 14 boys) with the

age range between 2 months and 5 years. The lot was divided in two groups: control group (15 subjects) and experiment group (15 subjects). The study subjects underwent physiotherapy and physical exercise and the experimental group followed a proprioceptive training program. The intervention consisted of three sessions of 45 minutes each, per week. The children were assessed before, during and after this physical intervention using Bayley Motor Scales of Infant Development.

**Results:** As the results indicated, the experimental group showed a statistically significant improvement in all measured values when compared with the control group.

**Conclusion:** The proprioceptive training was superior in the improvement of the posture, of the transfers and of the balance, compared to classical physical therapy. The delays in motor development milestones were more severe in the study group compared to experimental group.

#### P10.11

##### Antisense Oligomer (AO) induced exon skipping in the *mdx*<sup>Acv</sup> mouse model

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Duchenne muscular dystrophy (DMD), a relentless progressive muscular dystrophy is caused by protein truncating mutations in the dystrophin gene that result in the absence of functional dystrophin. Loss of dystrophin leads to irreparable membrane damage, thus promoting calcium ion influx and cell death.

Antisense Oligomer (AO) induced exon skipping is a molecular intervention whereby AOs are targeted to motifs involving in pre-mRNA splicing. This has been used to induce specific exon removal and by-pass the disease-causing gene lesion in the *mdx* mouse model of muscular dystrophy. We are investigating exon skipping in the B6Ros. Cg-Dmd<sup>mdx-4Cv/J</sup> (*mdx* 4<sup>Cv</sup>) muscular dystrophy mouse, which carries a nonsense mutation in exon 53 of the dystrophin gene. To restore the reading frame, both exons 52 and 53 must be excised from the mature dystrophin gene transcript to by-pass the primary gene lesion and maintain the reading frame. 2'-O-Methyl AOs, on a phosphorothioate backbone have been designed to mask these exons from the splicing machinery and lead to their excision. Initial AO combinations induced skipping of exons 52/53 and restored some protein expression. However the predominant transcript was missing only exon 53 as determined by RNA studies. We designed additional AOs to enhance exon skipping of both exons 52 and 53.

We highlight the importance of AO design to enhance efficiency of single and multi-exon removal.

#### P10.12

##### Study on the chaperone effect of several iminosugars and aminocyclitols on mutated glucocerebrosidases as a treatment for Gaucher disease

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Gaucher disease is an autosomal recessive disorder, characterized by the accumulation of glucosylceramide in lysosomes, because of acid β-glucosidase deficiency. Some competitive inhibitors, at subinhibitory concentrations, can work as chemical chaperones. We have tested the effect of two iminosugars, *N*-(n-nonyl)-deoxynojirimycin (NN-DNJ) and *N*-(n-butyl)-deoxynojirimycin (NB-DNJ), and four aminocyclitols on stable transfected COS-7 cells and patient fibroblasts.

In stable cell lines, NN-DNJ led to a 1.2 to 1.4-fold increase in the activity of G377S, N188S and wild-type GBAs at different concentrations. A slight increase was noticed in the activity of the N188S;E326K GBA at 2.5 μM. NB-DNJ at 5 μM induced a 1.2-fold increase in the GBA activity of COS-7 cells transfected with the N188S and N188S;E326K cDNAs. A slight increase was also observed in cells transfected with the wild-type cDNA. The aminocyclitol C4 Ph showed a 1.2 to 1.4-fold increase on wild-type and N188S GBAs, at different concentrations. At 15 μM, 1.2-fold increase for N188S;E326K GBA was also observed. In fibroblasts, treatment with NN-DNJ produced an increase of activity

in cells bearing the following genotypes: L444P;E326K/G202R (1.3-fold at 2.5  $\mu$ M), D409H/N188S;E326K (1.4-fold at 5 $\mu$ M and 10 $\mu$ M, and 1.2-fold at 20 $\mu$ M), and N370S/N370S (1.7-fold at 5 $\mu$ M and 10  $\mu$ M, and 1.5-fold at 20 $\mu$ M). Treatment with the aminocyclitol C10 at different concentrations increased 1.5-fold the GBA activity in L444P/G202R fibroblasts, and from 1.2 to 1.7-fold in L444P;E326K/G202R patient cells.

#### P10.13

#### Hematopoietic stem cells therapy and risk of graft versus host disease: A report from Iran

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Transplanted hematopoietic stem cells (HSC) and progenitor's cells can treat malignant and nonmalignant disorders, including immunological, gynecological, neurological, endocrinological and others pathologies and disorders. The transplantation of HSC that was not genetically identical (allogeneic) to that of the recipient resulted in an immunologic reaction by the donor lymphocytes against the recipient, causing inflammation of the target tissues, termed graft-versus-host disease (GVHD). GVHD is one of the major limiting factors in successful HSC transplantation. A wide range of host antigens can initiate graft-versus-host-disease, among them the human leukocyte antigens (HLAs). HLA-identical siblings or HLA-identical unrelated donors often have genetically different proteins that can be presented by MHC molecules to the recipient's T-cells, which see these antigens as foreign and so mount an immune response.

we enrolled 18 patients with one form of neuromuscular disorders at Genetics Department of Medical Special Center in Tehran. The all patients received transplants of HSC of the human embryonic liver. All patients were treated on clinical protocols, which were reviewed and approved by the Embryotech and Special Medical Centers. All patients provided writing informed consent before being enrolled in the protocols.

The diagnosis of acute GVHD was initially based on clinical signs must be confirm by positive biopsy results from at least one involved organ. In period of observation GVHD is developed in nobody. The lowest risk of GVHD is associated with that the HSC derived from liver do not contain the antigens of major histocompatibility complex on their surface what makes them tolerant towards recipients.

#### P10.14

#### Effects of silibinin on cell growth and invasive properties of a human hepatocellular carcinoma cell line, HepG-2, through inhibition of extracellular signal-regulated kinase 1/2 phosphorylation

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The purpose of the current study is to evaluate the effect of silibinin on human hepatocellular carcinoma HepG-2 cells. MTT assay, LDH release, Gelatin zymography, Griess reaction, Cell-based ERK 1/2 phosphorylation assay and quantitative real-time RT-PCR were employed to appraise the effect of silibinin on cell proliferation, cytotoxicity, metastatic potential, nitric oxide (NO) production, ERK 1/2 phosphorylation and activation in HepG-2 cells. Silibinin inhibited cell proliferation, matrix metalloproteinase 2 enzymatic activity, NO production and ERK 1/2 phosphorylation in a dose-dependent manner without exerting any cytotoxicity effect. In addition, an expressive increase in mRNA levels of Raf kinase inhibitor protein (RKIP), sprouty-related protein 1 with EVH-1 domain (Spred-1), sprouty-related protein with EVH-1 domain 2 (Spred-2) and tissue inhibitor of matrix metalloproteinase 2 (TIMP-2) coupled with a significant reduction in transcriptional levels of highly expressed in cancer (Hec1) and MMP-2 were observed. Altogether, these issues show for the first time that silibinin treatment could inhibit cell proliferation and invasive potential of HepG-2 cells through inhibition of ERK 1/2 cascade both directly (through suppression of ERK 1/2 phosphorylation) and indirectly (through up-regulation of RKIP, Spred-1 and Spred-2). In addition, cell growth and proliferation may be inhibited by silibinin through down-regulation of Hec1.

#### P10.15

#### Evaluation of transient transfection methods in Hu11 hybrid cells

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One important step in expression of exogenous genes in cells is transfection. The Hu11 cell line is a mouse erythroleukemia (MEL) cell line containing the human chromosome 11 (and thus the  $\beta$ -locus). Hu11 cells express the human globin genes and thus should be useful for studying the basis of globin gene regulation. In this study, we tested several transient transfection methods in Hu11 hybrid cells. The PSV- $\beta$ -Galactosidase vector was used as a control to monitor transfection efficiency. We tested commercially available reagents such as Lipofectamine<sup>TM</sup> 2000 (Invitrogen), FuGENE<sup>®</sup> HD Transfection Reagent (Roche), X-tremeGENE siRNA Transfection Reagent (Roche), HiPerfect Transfection Reagent (Qiagen) and DEAE-Dextran (Sigma). Also Hu11 cells were electroporated under different conditions. We have shown that different cationic lipid transfection reagents do not provide a reliable and effective method of transfecting Hu11 cells. Also electroporation did not work well for this cell line. Therefore we suggest that the best method for efficient transfection of Hu11 cells can be accomplished via a viral vector-based transfection procedure.

#### P10.16

#### Characteristics of patients with Hunter syndrome in Spain and Portugal compared with those in the rest of the world: analysis of data from HOS - the Hunter Outcome Survey

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Aims: To compare the characteristics of patients with Hunter syndrome (mucopolysaccharidosis type II) in Spain and Portugal with patients in the rest of the world (ROW).

Methods: Analysis of data from HOS - the Hunter Outcome Survey - was conducted in January 2008. HOS is a global outcome survey established to assess the natural history of Hunter syndrome and the safety and effectiveness of enzyme replacement therapy with idursulfase (Elaprase<sup>®</sup>; Shire HGT, Danderyd, Sweden). As of January 2008, there were 367 'prospective' patients included in HOS, 33 of whom were from Spain and Portugal.

Results: Mean age ( $\pm$ SD) at onset of symptoms in patients from Spain/Portugal was  $1.7 \pm 1.1$  years and from the ROW  $2.1 \pm 1.8$  years. Delay in diagnosis after symptom onset in Spain/Portugal was markedly less than that for the ROW ( $1.1 \pm 1.4$  vs  $2.2 \pm 2.9$ , respectively). The occurrence of any neurological signs/symptoms was similar (84%) in patients from Spain/Portugal and the ROW. Respiratory symptoms were reported in 75% of patients in Spain/Portugal and in 84% of patients from the ROW. Cardiovascular signs/symptoms were reported in 69% and 85% of patients from Spain/Portugal and the ROW, respectively. Characteristic facial features were the most commonly reported manifestation of Hunter syndrome, occurring in over 90% of patients in Spain/Portugal and the ROW.

Conclusions: This analysis of HOS data indicates no substantial difference between patients with Hunter syndrome in Spain/Portugal and the ROW. However, it highlights the delay between the occurrence of signs/symptoms and diagnosis, and the need for increased awareness of this rare disease.

#### P10.17

#### Optimization of transient transfection of K562 in order to siRNA transfection

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The K562 cell line is an erythroleukemia cell line that is widely used for studies of globin gene regulation. In this study, we have tested several transient transfection methods in K562 cells. The PSV-β-Galactosidase vector was used as a control to monitor transfection efficiency. We tested commercially available reagents such as Lipofectamin™ 2000 (Invitrogen), FuGENE® HD transfection Reagent (Roche), X-tremeGENE siRNA transfection Reagent (Roche), HiPerfect Transfection Reagent (Qiagen) and DEAE-Dextran (Sigma). Also K562 cells were electroporated under different conditions. Our Result demonstrated that Lipofectamin™ 2000 provide a reliable, effective and reproducible method for transfecting K562 cells. We have also used Lipofectamin™ 2000 to successfully transfet positive and negative control siRNAs into K562 cells. We are now in the process of using this system to study the effect of siRNAs against potential γ-globin repressors (in the hope of inducing the γ-globin gene).

#### P10.18

#### **Loeys-Dietz syndrome: In-vitro restoration of fibrillin and elastin production after treatment with losartan and dexamethasone**

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A 2-year-old boy born to non-consanguineous parents was seen by us following his fourth inguinal hernia repair. He was noted to have facial dysmorphism including brachycephaly, high forehead, hypoplastic supraorbital ridges and malar areas, proptosis with ptosis, posteriorly rotated ears, high arched palate with a normal uvula, arachnodactyly and camptodactyly. Echocardiography demonstrated a dilated aortic root and tortuous aortic arch and branches. X-rays revealed craniocervical instability, craniosynostosis and generalized osteopenia. Loeys-Dietz syndrome (LDS) was suspected clinically and DNA analysis of the *TGFBR1* gene revealed a missense mutation in codon 4 (c.722C>T) leading to substitution p.S241L. No mutations in the *TGFBR2* or *FBN1* genes were detected.

*In vitro* studies of skin fibroblasts derived from this patient indicated that they were significantly deficient in elastin and fibrillin gene expression (RT-PCR technique). Consistently, immunohistochemistry confirmed a lack of adequate production of elastin and fibrillin. Production of fibulins 1, 2 and 5, auxiliary components of elastic fibers shown to be important in normal elastogenesis, was not affected. Treatment of the cultured fibroblasts with losartan (angiotensin II receptor blocker) or dexamethasone restored normal production of elastic and fibrillin fibers. *In vitro* treatment of fibroblasts derived from other LDS patients is underway to assess the reproducibility of these findings and may provide more information on the potential for future clinical use. Our findings further highlight the overall role of imbalanced signal transduction through TGF receptors that lead to impaired elastogenesis in LDS and similar diseases.

#### P10.19

#### **Towards a pharmacological therapy for Mandibuloacral Dysplasia syndrome**

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Recently, different groups have demonstrated that the farnesyl transferase inhibitors (FTIs) were able to reverse *in-vitro* and *in-vivo* some phenotypic manifestations of progeroid syndromes secondary to *LMNA* and/or *ZMPSTE24* mutations (Hutchinson-Gilford progeria syndrome and restrictive dermopathy). The rationale of this treatment is based on blockage of the toxic effect of the farnesylated forms of prelamin A which in turn is responsible of cellular morphology alterations and genomic instability. In order to verify if this treatment is reproducible also in the mandibuloacral dysplasia (MADA), we studied the cellular effects of FTIs treatment on primary fibroblasts cell lines after 72 hrs at different concentrations (10 - 500 nM) of the drug. We observed that this treatment induces in MADA's cells, an increase of abnormal nuclei in a dose-manner dependent effect. On the basis of these results, we

decided to test the effect of two different drugs (bisphosphonates and statins) known to act on the same biochemical pathway at different levels. We treated MAD fibroblasts in a two steps model (24 hrs statins treatment and then 12hrs bisphosphonate in a single dose). This treatment, showed an improvement of the cellular phenotype (reduction of the number of misshapen nuclei and a partial rescue of the heterochromatin organization). Singularly, these drugs were ineffective. All together these results, suggest that FTI treatment is ineffectiveness versus MADA patients, while inhibitors of prenylation pathways could be considered as potential.

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#### P10.20

#### **A tendency in the treatment of mitochondrial complex I deficiency**

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The treatment of the mitochondrial disorders is difficult and not well known. With an integral clinical-laboratory method were diagnosed 5 children with mitochondrial complex I deficiency: 2 patients with mutation of ND5 gene and 3 - with ND3 , ND2, ND1 . Their mutations were confirmed by PCR SBT method of mitochondrial regions in peripheral intravenous blood. The clinical symptoms were various .The child with ND5 mutation- T 12880 C (with aminoacid change phenylalanine with leucine) had hemiparesis, weakness , muscle hypotonia, decreased sensitivity, the other boy (synonym basal changes), T 11311 C and polymorphisms- convulsions, ophthalmoplegia, ataxia ,decreased hearing and vision, weakness and fatigability. The patient with ND3 mutation had the same symptoms, but more severe: atrophy of n. opticus , deafness due to neuritis nervi acustici and severe ataxia. The child with ND2 mutation C 5472 G and multiple polymorphisms had muscle hypotonia, hyporeflexia, myoclonic convulsions. After treatment with high doses vitamins B1, B2, B6, B12, Q10, L-carnitine the general condition and neurologic status improved. The children with ND5 mutation recovered at all , as the patients with ND 3 and other mutations who showed an improvement too. The treatment of the children proceeds.

#### P10.21

#### **Indicating FTA Elute: for the collection, processing, and elution of DNA from biologically clear samples for use in downstream amplification technologies**

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Collecting samples using an oral swab and Indicating FTA Elute is a non-invasive procedure that can be carried out easily and safely by the layperson, thus providing an ideal format for collecting human genetic material from virtually anywhere in the world.

To visualize sample collection and placement, Indicating FTA Elute has been developed for use with clear biological samples by the incorporation of a colour indicating dye that distinguishes the clear sample once it is applied to the matrix. It is functionally equivalent to traditional FTA Elute in that it protects DNA samples from degradation and provides a source of amplifiable DNA which is eluted from its matrix with a simple heat and water elution step. We ran a number of tests on various biological samples to show that DNA eluted from Indicating FTA Elute is functionally identical to that eluted from traditional FTA Elute. The aim was to demonstrate that Indicating FTA Elute provides a template for amplification dependent assays such as STR and allelic discrimination analysis but greatly simplified and optimized sample extraction from the matrix.

In summary, Indicating FTA Elute in combination with an oral swab represents a revolutionary, non-invasive method for simplified genetic sample collection, sample transport in the mail, simplified DNA extraction requiring nothing more than water and heat, plus the added benefit of storing the samples at room temperature for literally years if this is a requirement. Indicating FTA Elute is therefore perfectly positioned to support the emerging discipline of pharmacogenomics or personalized medicine.

**P10.22****Oxidative stress in nonsyndromic sensorineural hearing loss**H. T. El-Bassyouni<sup>1</sup>, A. M. Ashour<sup>1</sup>, M. Afifi<sup>2</sup>, A. Ezzat<sup>1</sup>;<sup>1</sup>National Research Center, Guiza, Egypt, <sup>2</sup>Hearing and speech Institute, Guiza, Egypt.

**Introduction:** Ototoxicity seems to result from the inhibition of cochlear antioxidant defenses, causing an increase in the amount of reactive oxygen species. These findings suggest a causal relationship between the formation of reactive oxygen species, oxidative stress and functional/morphological ear damage.

**Aim:** to shed more light on the link between oxidative stress and non-syndromic sensorineural hearing loss.

**Methods:** auditory function was monitored along with plasma concentrations of copper, zinc, calcium, phosphorus, magnesium and iron. Further more, lipid peroxidation, β carotene, vitamin A, E and C activities and immunoglobulin G, M and A levels were estimated.

**Results:** Significant decrease in calcium, phosphorus, β carotene, and vitamin E activities were found, as well as low levels of immunoglobulin G and M. Increase in lipid peroxidation was observed indicating oxidative stress which suggests a putative role of antioxidants in the pathogenesis of sensorineural hearing loss.

**Conclusion:** The results support the hypothesis that dietary and immune factors influence individual susceptibility to hearing loss. Further studies are needed to verify whether antioxidants, correction of deficient nutrients and/or immune modulation would improve sensorineural hearing loss.

**P10.23****Role of amniotic fluid mesenchymal stem cells in gene and cell therapy**F. Bolda<sup>1</sup>, A. Bos<sup>2</sup>, R. Baffell<sup>2</sup>, G. Porta<sup>3</sup>, W. Quasim<sup>4</sup>, B. Gaspar<sup>4</sup>, F. Porta<sup>2</sup>, A. Lanfranchi<sup>2</sup>;<sup>1</sup>Stem Cell Lab, Oncohaematology and BMT Units, Ospedale dei Bambini, brescia, Italy, <sup>2</sup>Stem Cell Lab, Oncohaematology and BMT Units, Ospedale dei Bambini, Brescia, Italy, <sup>3</sup>Università dell'Insubria, Varese, Italy, <sup>4</sup>Department of Clinical Immunology, Great Ormond Street Hospital NHS Trust, London, United Kingdom.

Mesenchymal stem cells (MSCs) are multipotent cells capable of differentiating into diverse lineages: osteocytes, chondrocytes, adipocytes, neuronal cells and cardiomyocytes. MSCs were initially identified in adult bone marrow (BM), but cells resembling BM-MSCs have also been found in many other tissues: adult and foetal peripheral blood, foetal liver, foetal spleen, placenta, umbilical cord, amniotic membrane and synovial fluid.

Human amniotic fluid (HAF) obtained during the process of amniocentesis is a valid source of MSCs and they could be a valid vehicles for gene therapy.

The aim of this study was to isolate MSCs from amniotic fluid and to test their transduction efficiency.

HAF specimens were obtained between 15 and 18 weeks' gestation by amniocentesis, previous written consent.

Amniotic-fluid MSCs (AFMSCs) were cultured under specific conditions for 7 weeks and analysed by flow cytometry and quantitative real time PCR to assess the presence and the expression levels of specific markers. Mesenchymal markers (CD73, CD106, CD54, CD90, CD29, CD44, CD105) present a peak of expression between 3<sup>rd</sup> and 4<sup>th</sup> week of culture.

To assess the transducibility of AFMSC we used a SIN HIV-1-based lentiviral vector. This vector encloses SFFV-U3 promoter and eGFP. It also encodes a mutant WPRE and cPPT.

Transduction efficiency was 60%. We demonstrate with the immunoistochemistry and molecular techniques the presence of human multipotent MSCs in the second-trimester amniotic fluid. We suggest that AFMSCs may be good target for prenatal gene therapy. The model under investigation is severe combined immunodeficiency (SCID) due to adenosine deaminase deficiency (ADA).

**P10.24****BNP is a transcriptional target of the short stature homeobox gene SHOX**A. Marchini<sup>1</sup>, B. Häcker<sup>1</sup>, T. Marttila<sup>1</sup>, V. Hesse<sup>2</sup>, J. Emons<sup>3</sup>, B. Weiß<sup>1</sup>, M. Karperien<sup>3</sup>, G. A. Rappold<sup>1</sup>;<sup>1</sup>Institute of Human Genetics, Heidelberg, Germany, <sup>2</sup>Dept. of Pediat-

rics, Sana Klinikum Lichtenberg, Berlin, Germany, <sup>3</sup>Dept. of Pediatrics & Endocrinol.&Metab. Diseases, Leiden Univ. Med. Center, Leiden, The Netherlands.

Short stature due to SHOX deficiency represents a common congenital form of growth failure and is involved in the etiology of "idiopathic" short stature and the growth deficits and skeletal anomalies in Léri-Weill, Langer and Turner syndrome. While much is known on the clinical and molecular aspects of SHOX haploinsufficiency, the integration of SHOX in the signalling pathways regulating bone growth is currently not defined. Here we identify *NPPB* encoding the natriuretic peptide BNP, a well known approved cardiac and natriuretic peptide hormone (drug), as a transcriptional target of SHOX. The ability of SHOX to transactivate the *NPPB* endogenous promoter was demonstrated in luciferase reporter assays using serial deletions of the *NPPB* promoter region. Binding of SHOX to the *NPPB* promoter was also demonstrated *in vivo* by chromatin fixation and immunoprecipitation. We also demonstrate the lack of promoter activation in two SHOX mutants from patients with Léri-Weill syndrome. In addition, immunohistochemical analysis of human growth plate sections showed for the first time a co-expression of BNP and SHOX in late proliferative and hypertrophic chondrocytes. Together these data strongly suggest that BNP represents a direct target of SHOX.

One may speculate that BNP as a downstream effector of SHOX may also open up new potential avenues for the treatment of short stature. BNP in the systemic circulation is likely to reach growth plate chondrocytes. It may either directly influence NPR-B signaling or indirectly increase local CNP levels by saturating the competing receptor NPR-C.

**P10.25****Designing and constructing a new package for further suicide gene therapy**S. Hajizadeh-Sikaroodi<sup>1</sup>, S. Zeinali<sup>2</sup>, A. Kayhan<sup>3</sup>, M. Jafarpour<sup>2</sup>, L. Teimoori-Toolabi<sup>2</sup>;<sup>1</sup>Research and Science Azad University, Tehran, Islamic Republic of Iran,<sup>2</sup>Department of molecular medicine, Pasteur Institute of Iran, Tehran, Islamic Republic of Iran, <sup>3</sup>Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Islamic Republic of Iran.

A commonly used strategy for gene therapy of solid tumors is suicide gene therapy. In order to decrease the side-effects of suicide gene therapy it would be better to express the suicide genes selectively in cancerous tissues. Using cancer specific promoters is the most applicable strategy to reach this aim. CD (Cytosine Deaminase) and TK (Thymidine Kinase) are used as suicide genes, but a suitable vector is needed for inserting any promoters upstream of them.

Firstly, the new MCS was designed by studying the sequence of our own pUClacZ (engineered pUC which can express LacZ in eukaryotic cells because of its BGH-polyA downstream of LacZ). Two partial complementary 37 base long oligonucleotides were designed.

Cytosine deaminase and thymidine kinase genes were amplified by PCR on *saccharomyces cerevisiae* and *herpes simplex virus* DNA, and then were inserted downstream of MCS instead of lacZ.

Insertion of the MCS bidirectionally into pUClacZ was proved by PCR and digestion analysis. In fact we made six constructs which are capable of adopting suitable and specific promoters upstream of these two suicide genes and LacZ reporter gene.

pUC-RMCS-TK

pUC-RMCS-CD

pUC-FMCS-TK

pUC-FMCS-CD

pUC-LacZ-CD

pUC-LacZ-TK

These constructs are compatible plasmids for further tumor specific suicide gene therapy as the availability of suitable restriction sites in MCS enables us to clone any promoter directionally upstream of reporter and suicide genes. These reporter and suicide constructs can be used in gene therapy of any types of cancer by the potential of accepting any tumor specific promoter upstream of their genes.

**P10.26****Treatment of SMA fibroblasts and lymphoblasts with drugs that increase SMN expression reveals no responders and no additive effects**

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Spinal muscular atrophy (SMA) is a neuromuscular genetic disorder caused by mutations in the SMN1 gene. The homologous copy (SMN2) is always present in SMA patients. Exon 7 is spliced out of most SMN2 transcripts (delta7) whereas SMN1 gene transcripts contain mainly exon 7 (full-length). Recent advances in SMA treatment with pharmacologic agents have resulted in an increase in SMN mRNA and protein levels *in vitro*. We performed a systematic analysis of SMN expression in primary fibroblasts and lymphoblasts from 7 SMA patients with varying clinical severity and different SMN1 genotypes to determine expression differences in two accessible tissues (skin and blood). The basal expression of SMN mRNA full-length and delta7 in fibroblasts and lymphoblasts at 24 and 48 hours was performed by quantitative real time PCR. The ratio SMNFL/SMNΔ7 was significantly higher in control cells than in patients ( $p<0.001$ ). The FL/Δ7 ratios ranged between 0.8-1.53 in SMA fibroblasts whereas they were between 1.18-2.82 in lymphoblasts. Furthermore, we investigated the response of these cell lines to different drugs such as valproic acid, phenylbutyrate and hydroxyurea. We classified the samples according to the increase in SMN expression as responders or non responders (to one or more of the drugs). Moreover, the combination of two of the drugs showed no synergistic effect in the responder cases. Despite the similarity of the basal SMN expression in our cells, the results of treatments that increase SMN expression was very variable, suggesting individual response factors. Supported by FIS05-2416, CIBERER and Proyect GENAME.

**P10.27****Oxidative stress damage as early event in the pathogenesis of X-Adrenoleukodystrophy: therapeutic implications**

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X-linked adrenoleukodystrophy (X-ALD) is a fatal neurodegenerative disorder, characterized by progressive cerebral demyelination (CCALD) or spinal cord neurodegeneration (adrenomyeloneuropathy, AMN), adrenal insufficiency and accumulation of very long-chain fatty acids (VLCFA) in tissues. The disease is caused by mutations in the ABCD1 gene, which encodes a peroxisomal transporter that plays

a role in the beta-oxidation of VLCFA into peroxisomes. The *Abcd1* knock-out mice develop a spinal cord disease that mimicks AMN in adult patients, with late onset at 20 months of age. The mechanisms underlying cerebral demyelination or axonal degeneration in spinal cord are unknown. Here, we present evidence by GC/MS that MDAL (malonaldehyde-lysine), a consequence of lipoxidative damage to proteins, accumulates in the spinal cord of *Abcd1* knockout mice as early as 3.5 months of age. At 12 months, *Abcd1*- mice accumulate additional proteins modified by oxidative damage arising from metal-catalyzed oxidation and glycoxidation/lipoxidation. While we show that VLCFA excess activate enzymatic antioxidant defences at the protein expression levels, both in neural tissue, in *ex-vivo* organotypic spinal cord slices from *Abcd1*- mice and in human ALD fibroblasts, we also demonstrate that the loss of *Abcd1* gene function hampers oxidative stress homeostasis. We find that the α-tocopherol analog Trolox is able to reverse oxidative lesions *in vitro*, thus providing therapeutic hope. These results pave the way for the identification of therapeutic targets that could reverse the deregulated response to oxidative stress in X-ALD.

**P10.28****Recombinant lentiviral particles for gene therapy of β-thalassemia**

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β-thalassemia disease is caused by absence or reduction of β-globin chain synthesis. The β-globin gene is controlled by LCR. Gene therapy using homologous recombination is method of choice for replacing the defective beta-globin gene with the normal one. One of the effective therapeutic methods for this disease is gene therapy by lentiviral vectors.

The capacity of lentiviral vectors is approximately 9 kb. The transgene was designed as a 6 kb construct containing mini LCR and β-globin gene. HS2, HS3, HS4 segments (mini LCR) and β-globin gene with 5' and 3' UTR amplified from the genomic DNA from a normal individual by PCR. Each of segment was cloned into pTZ57R/T vector and then sub cloned in pBGGT vector. Finally this construct was subcloned into a lenti transfer vector and confirmed by restriction digestion and sequencing. Transfer vector and three packaging plasmids (Plp1, Plp2, Plp/VSVG) were cotransfected into 293T packaging cell line by the use of lipofectamine 2000. Presence of transgene was confirmed in the harvested viruses by RT PCR on extracted RNA from these recombinant lentiviruses and also by p24 ELISA test. The titer of lentiviral stock was determined in a HT1080, K562 cell lines and compared with COS-7 cell line. This titer, in HT1080 cell line, was higher than COS-7 and K562. The remained transduced COS-7 colonies were expanded and DNA was extracted. PCR was used to evaluate random integration of construct in the genome and compared with homologues recombination in this gene transfer technique.

## EMPG PLENARY LECTURES

### EPL1.1

#### Recall and interpretation in genetic counselling, after intake and non-informative DNA-test results in BRCA1/2: discrepancies, explanations and consequences

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**Background** The patients' subjective interpretation of genetic counselling may be as important as correct understanding.

**Methods** In study I, 166 cancer patients filled-in a questionnaire after intake for hereditary breast/ovarian cancer. To date, 31 of them received an inconclusive (no-mutation) DNA-test result and filled-in a second questionnaire. In study II, 24 cancer patients were interviewed 1-3 years after receiving an Unclassified Variant-result (i.e. variant-of-uncertain-clinical-significance).

**Results** In study I, although most counselees correctly *recalled* the communicated medium heredity and intermediate/high cancer risks, they *interpreted* this like pathogenic information. After inconclusive results, counselees correctly *recalled* the counsellor communicating a non-informative DNA-result with unchanged cancer risks. However, heredity was *recalled* as low and *interpreted* as medium.

In study II, sixty-seven percent *recalled* having received a non-informative DNA-result, but 80% *interpreted* this as pathogenic.

In both studies, discrepancy between *interpretation* and *recall* were explained by previous interpretations, health history, genetic uncertainty, denial coping style, autonomy, self-acceptance, low need for certainty.

In both studies, *interpretation* explained changes in intended and actual cancer screening and surgery; in study I, *recall* explained some of these changes.

**Discussion** Counselees subjectively interpreted intake information and non-informative DNA-results as being more pathogenic than how they factually recalled counselling sessions.

Interpretation explained more than recall. Previous interpretations, health, and personality explained recall-interpretation discrepancies. The decrease in recalled/interpreted heredity after inconclusive results is probably due to correction of pathogenic interpretation at intake, and need for certainty. Counsellors and researchers should address discrepancies between the counselees' recall and interpretation of genetic information.

### EPL1.2

#### Is enhanced information at genetic counselling necessary? A randomised study

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**Purpose:** The aim of this randomized study was to explore the effect of receiving enhanced onco-genetic information on probands' knowledge, risk perception, distress and communicating information to at-risk relatives.

**Patient and methods:** A total of 148 subjects (134 female and 14 male) who attended genetic counselling at the onco-genetic clinic of Uppsala University Hospital, Sweden (2003-2006) were randomised to information (n=85) or control (n=78) groups. The intervention group received the videotaped session, a copy of medical records, pedigree and a pamphlet about basic genetic concepts. Additionally, they had contact with a specialist nurse for more discussion and further questions. The control group received the standard information and the video in association with last follow-up eight month later. The knowledge about hereditary cancer, levels of emotional distress and personal risk estimations were measured at the base line, two weeks and eight months later.

**Results:** The majority had a high level of knowledge about hereditary

cancer, although a significant improvement was observed in breast cancer group after counselling. The levels of anxiety and depression indicated a significant reduction at follow-ups. At tow-weeks follow-up, the correct estimation of personal risk for developing hereditary cancer, compared to initial estimation pre-counselling, had changed from 47% to 90% in the intervention and from 45% to 85% in control group. The majority (86% in control and 95% in intervention group) intended to communicate the genetic information to their relatives.

**Conclusion:** Enhanced information did not improve the participants risk perception. However the intention of informing relatives was elevated.

### EPL1.3

#### Determining the genetic status of women newly diagnosed with breast cancer prior to treatment decisions: An ethical challenge

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**Background:** Ethical issues raised in genetic consultations for common diseases, for example, confidentiality and the disclosure of genetic information from bioethical, legal and user perspectives have been documented. However, less is known about cancer genetics service providers' experience of ethical issues that arise in their practice. **Aims:** This study aimed to document the types of issues practitioners define as ethically contentious in their practice, with what frequency they occur, or how they attempt to resolve them. **Method:** We undertook semi-structured telephone interviews with 21 genetic counsellors and 13 clinicians. All interviews were tape-recorded, transcribed verbatim and a thematic analysis was undertaken. **Results:** BRCA1/2 mutation testing to tailor the management of newly diagnosed high risk women was identified as a future ethical challenge for cancer genetics services. Themes identified included balancing the ethical concept of "do no harm" with the rights of women to be fully informed prior to making treatment decisions; questioning the ability of a woman to fully comprehend the implications of a positive mutation test on herself and other family members and managing the increasing client load on limited clinic resources. The importance of involving genetic counsellors in the multi-disciplinary team was emphasised. Clinicians identified fewer barriers to fast tracking BRCA1/2 testing than did genetic counsellors. **Conclusion:** Further understanding of the barriers to the management of BRCA1/2 testing in newly diagnosed breast cancer patients is needed to guide future cancer genetics practice as clinical testing is likely to transform the direction, priorities and processes of cancer genetic services.

### EPL1.4

#### Impact of using family history on motivation to prevent type 2 diabetes: an RCT

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Genetic susceptibility testing for many multifactorial diseases is not yet warranted. Family history is an important risk factor for many common chronic diseases that may be used as a surrogate marker for genetic susceptibility. The aim of this study was to assess the potential effectiveness of communicating familial risk of type 2 diabetes and emphasising preventive options, on intentions to change behaviour to reduce the risk.

Individuals with a positive family history of diabetes were randomised into two groups: risk information based on general risk factors and familial risk (n=54) (intervention group) or risk information based on general risk factors alone (n=54) (control group). The information was provided during a personal consultation. Intentions to change health behaviour, self-reported behaviours (healthy diet; physical activity), perceived causes and severity of diabetes, perceived controllability,

and perceived risk were assessed using questionnaires (at baseline, one-week, and three-months follow-up).

Compared with those receiving information based on general risk factors alone, those receiving information on familial risk perceived heredity to be a more important cause of diabetes ( $p<0.01$ ) and greater control over developing diabetes ( $p=0.001$ ). Although at one-week follow-up there were no differences in intentions to change behaviour between the two groups, at three-months follow-up the intervention group reported having eaten more healthily ( $p=0.01$ ).

Familial risk information given in a personal consultation did not result in fatalism. There was some evidence to suggest it increased healthy behaviour. More research is needed to confirm these findings in a larger sample, using objective measures of health-related behaviours.

#### EPL1.5

##### **Illness risk representations and preventive behaviour in people diagnosed with Familial Hypercholesterolemia by DNA-testing**

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The objective of the study was to explore illness risk representations and preventive behaviour of people diagnosed with Familial hypercholesterolemia by DNA-testing.

The study employed a cross-sectional design, using Leventhal's Common Sense Model of self-regulation of health and illness as a framework, and was carried out among people recently diagnosed with FH by DNA-testing. Illness risk representations and preventive behaviour of 81 participants were assessed by self-administered postal questionnaires.

A majority of participants (75%) perceived their risk of developing CVD as lower or equal to that of an average person their age. Although in general participants believed strongly in the efficacy of the recommended actions, medication was seen as being somewhat more effective than a healthy diet and physical activity in reducing the risk of CVD ( $t(65) = 2.460$ ,  $p=.017$ ). Of hypercholesterolemics, 88% reported using cholesterol-lowering drugs as prescribed, whereas only 50% of all participants reported following recommendations concerning diet and physical activity. 70% reported having one or more first-degree relatives with CVD. Participants with more affected family members had higher risk perceptions and were more likely to perceive CVD as a permanent condition, consider hereditary as a likely cause of CVD, and report following recommendations concerning diet and physical activity.

In conclusion: In general FH screened positives are optimistic about their risk of developing cardiovascular disease. Those with more first-degree family members affected with CVD are less optimistic about their risk and more likely to adopt a healthy diet or to be physically active.

#### EPL2.1

##### **Genetic testing in asymptomatic minors: social and ethical issues**

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Background. Although various guidelines and position papers have discussed in the past the ethical aspects of genetic testing in asymptomatic minors, the European Society of Human Genetics had not endorsed any statement exclusively focused on this issue.

Objective. Within the project Eurogentest attention has been devoted to the provision of appropriate counselling to accompany genetic testing, the education of patients and professionals, and the ethical, legal and social issues surrounding testing. The focus of the ethics unit was oriented towards the study of the ethical issues related to genetic testing in minors.

Method. Firstly, research has been performed on the existing recommendations regarding predictive genetic testing in minors and carrier testing, with as main attention to identify areas of agreement and disagreement. Secondly, the medical-ethical and medical-legal literature regarding predictive genetic testing in minors, carrier testing, the position of minors and patient rights was studied. Thirdly, a systematic literature review has been realized to gather information regarding the

attitudes of different stakeholders (minors, healthcare professionals, parents and relatives of affected individuals) towards genetic testing in asymptomatic minors. Fourthly, the attitudes of European clinical geneticists were gathered with regard to genetic testing in asymptomatic minors.

Results. Based on this preparatory work a draft guideline and background paper were elaborated. These will be discussed with the Professional and Public Policy Committee of the European Society of Human Genetics. Afterwards, both documents will be sent out for public consultation.

#### EPL2.2

##### **Public attitudes towards genetic testing for susceptibility to major depression**

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Introduction: Successful intervention in psychiatry based on genetic risk will depend on public understanding of and attitudes towards the complexity of risk prediction involving susceptibility genes and gene-environment interactions. This study aimed to qualitatively evaluate attitudes and beliefs regarding the psychosocial implications of genetic risk for major depression in an urbanised population.

Method: Participants (N=36) of focus groups discussed their understanding of the role of genes in psychiatric disorders and their beliefs and attitudes towards genetic testing for risk for major depression.

Results: More than half the participants reported personal or family history of psychiatric disorders. The majority (67%) of participants indicated interest in having a genetic test for susceptibility to major depression, if it was available. After discussion of perceived psychosocial implications, only 42% still favoured having the test. All participants still interested in genetic risk testing for major depression reported they would only do so through trusted medical professionals. Participants were unanimously against independent genetic testing via the internet.

Discussion: Having a family history of psychiatric disorders was a major reason for interest in having a genetic test to determine risk for depression, which participants reported would enable them to be better prepared. Psychosocial implications influencing change of attitude included privacy issues and fear of genetic discrimination by insurance companies and employers.

Conclusion: The study highlights the importance of public education about psychiatric genetics, potential risks and benefits of genetic testing and availability of appropriate support services in association with future genetic risk testing in psychiatry.

#### EPL2.3

##### **Exploring Psychiatric Genetics through Interactive Drama**

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The Wales Gene Park recently collaborated with the Genetic Interest Group and Gwent Theatre to develop and produce an interactive theatre workshop exploring contemporary developments in human genetics for young people from schools and colleges across Wales. The collaboration combined expertise in scientific and social and ethical aspects of genetics, science communication and in developing drama techniques for education. The main aim of the project was to use an innovative and creative approach to engage young people and encourage understanding and appreciation of different aspects of a complex area of biomedical science and the associated social and ethical issues. The project was funded by a Wellcome Trust Public Engagement Society Award.

Working with a writer, the project team developed a challenging drama centred on a young man's experience of mental illness. The performance explored questions and issues related to genetics and mental health including those surrounding family relationships, expectations of 'normality' and social responsibility. The young people's participation was task driven and structured to facilitate an intellectually and emotionally stimulating learning experience, requiring them to take an investigatory and mediatory role in the drama. Additional educational resources for use in school extended the learning experience beyond

the activity itself.

This presentation draws on material produced by participants during the development of the project and subsequent performances to illustrate how aspects of the methodology used within the project sought to engage participants and to facilitate their appreciation complexity of the issues raised.

#### EPL2.4

#### Professional ambivalence: accounts of ethical practice in childhood genetic testing

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Childhood genetic testing raises complex ethical and moral dilemmas for both families and professionals. In the family sphere, the role of communication is a key aspect in the transmission of 'genetic responsibility' between adults and children. In the professional sphere, genetic responsibility is an interactional accomplishment emerging from competing views over what constitutes the 'best interests' of the child. In the present paper we extend our previous research into parental accounts of childhood genetic testing and explore the ethical explanations/descriptions of professionals in research interviews. Interviews (n=20) were conducted with professional practitioners involved in the genetic diagnosis and management of children and their families. We first identify four inter-related themes - juxtaposition of parental rights vis-à-vis child's autonomy, elicitation of the child's autonomy, avoidance of parental responsibility and acknowledgement of uncertainty - and then, using Rhetorical Discourse Analysis, examine the range of devices through which ethical explanations are situationally illustrated: contrast, reported speech, constructed dialogue, character and event work. An important device for facilitating ethical explanations is the use of *extreme case scenarios* which reconstructs dilemmas as justifications of professional conduct. Our analysis of professional accounts suggests that ethical practice is not a simple matter of implementing principles but managing the practical consequences of interactions with parents and children. We conclude that more attention is needed to understand the way professional practitioners construct and share cases as useful illustrations of evidence-based ethical practice.

#### EPL3.1

#### Patients' attitudes to the use of preimplantation genetic diagnosis for inherited predispositions to bowel cancer

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Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are dominantly inherited cancer predisposition syndromes which confer a significantly increased risk of bowel and other cancers. The Human Fertilisation and Embryology Authority (HFEA) have approved preimplantation genetic diagnosis (PGD) for FAP and HNPCC in the UK. Semi-structured telephone interviews were used to explore the views of 6 FAP and 8 HNPCC mutation carriers (6 men and 8 women) regarding the use of PGD for their condition and other scenarios. The general acceptability of the use of PGD depended on the perceived severity of the condition in question and views about the moral status of the embryo/foetus during development. While both patient groups perceived FAP or HNPCC to have a low impact on quality of life, approximately half of the participants accepted the use of PGD for their condition in principle. Fewer participants however, from both the FAP and HNPCC groups, would consider personally using PGD for their condition. The importance of individual choice around reproductive decision-making was a central theme although there were concerns about the expansion of PGD into non-medical trait selection. The main advantage of PGD was seen to be avoiding termination of pregnancy. Disadvantages identified included the emotional and financial costs of undergoing the procedure, and the relatively low chance of a successful pregnancy.

#### EPL3.2

#### Pre-implantation genetic diagnosis (PGD) and prenatal diagnosis (PND) for cancer predisposition syndromes: reported practices and attitudes of French professionals

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PGD and terminations of pregnancy for foetal indications are registered in France but the spontaneous demand experienced by medical professionals in their practice is unknown.

Objectives : to describe the reported demand and acceptability of PGD/PND for cancer predisposition syndromes such as observed by cancer geneticists and prenatal diagnosis centres.

Methods: Mail/postal survey (self-administered questionnaires) among all French cancer geneticists (N=123) and multi-disciplinary prenatal diagnosis (MPD) teams (N=47) registered by the French regulatory biomedicine agency.

Results: Cancer geneticists (47 y.o , SD=8) and MPD team coordinators (55 y.o, SD=7) answered in 62% and 64% of the cases, respectively. Half the cancer geneticists reported a spontaneous demand from their consultees during the preceding year (50% for PGD, 59% for PND). The issue of PGD, had been raised for the same pathologies as for PND but less frequently. PND was discussed for Familial Adenomatous Polyposis (n=57), Retinoblastoma (n=45), NEM1 (n=34), Breast-Ovarian Cancer (n=20) and Li-Fraumeni syndrome (n=8). MPD teams only reported 6 requests for PGD (5 for FAP; 1 for BRCA1). When cancers are likely to occur during early childhood with high penetrance, high severity and no effective prevention/treatment, the acceptability of PGD/PND was very high (>80%). When cancers are likely to occur before 50 y.o. but never in childhood and that an effective non deleterious prevention/treatment is available, the acceptability of both interventions was low but much higher for PGD (40%) than for PND (<27%) .

Conclusion: Spontaneous demand for PGD/PND for cancer predisposing syndromes is already an issue in France.

#### EPL3.3

#### Motivations for genetic testing among individuals at risk of hereditary breast-ovarian cancer (HBOC) and Lynch syndromes

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Background: Knowing the motivations for genetic testing may help address pre-test genetic counselling sessions more individually.

Methods: Individuals (n=292) undergoing genetic testing for HBOC and Lynch syndrome who had received genetic counselling were included in a prospective multicenter study (IMASS).Individuals completed a self-reported questionnaire about demographic and clinical information,psychological well-being and motivations about genetic testing.Chi-square test was used to compare clinical and demographical variables with the main motivation for genetic testing.

Results: Overall,240 individuals (82%) were women,median age= 44y (18-88).One hundred and eighty-one (62%) had been diagnosed with cancer,236 (81%) were at risk of HBOC,189 (62%) had children,154 were probands (65%), and 125 (43%) had the perception of being a mutation carrier.The three main reasons to undergo genetic testing were:to know if their children were at risk in 134 (46%),to know their cancer risk in 90 (31%),and to know if they need more screening practices in 40 (14%).

A greater proportion of individuals >30 y, and those affected by cancer,or having children,or probands reported that knowing if their children were at risk was the main reason to undergo genetic testing ( $p> 0.001$  in all cases),while individuals <30y, and those non-affected by cancer,not having children, and relatives undergoing testing of a

mutation identified in the family reported knowing their own risk as the main reason ( $p<0.001$ ).

**Conclusions:** The main motivation reported by individuals undergoing genetic testing is to know if their children are at risk. Pre-test genetic counselling sessions need to address specific motivations and concerns reported by individuals being tested.

#### EPL3.4

#### Exploring the influence of the family social network on the psychological well-being of individuals undergoing genetic counseling and testing for Lynch syndrome

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Facilitating family communication is an important component of genetic counseling. However, little is known about how the information obtained through the genetic counseling process is translated and communicated to family members. Furthermore, we do not know the effect of family conversations on the cognitive and social outcomes of other family members.

The current study focuses on families (240 persons representing 35 unrelated families) known to carry a mutation in a mismatch repair gene associated with Lynch syndrome. We investigated whether previous family experiences with genetic services and communication within the family network, are associated with a decline or increase in psychological distress or cancer worry. Dependent variables examined included distress, depressive symptoms, and cancer worries. Independent variables included 1) time (days) between provision of genetic test results to the proband and the provision of genetic counseling to family members at risk to inherit the mutation and; 2) the generation of the family member relative to the proband (older, same, or younger). Results indicate an increase in depressive symptoms ( $p = .06$ ) and cancer worry ( $p=.01$ ) with increasing time from the probands' notification. Family members in the same generation ( $p < .01$ ) as the proband or previous generation ( $p<.01$ ) experienced a significant increase in distress with increasing time from the probands' notification; however, those in younger generations experienced a significant decrease in distress ( $p=.03$ ).

Gaining insight about social influence processes will provide critical information for developing innovative genetic and genomic-based education & counseling programs that target families.

#### EPL3.5

#### Genetic counselling and testing for melanoma risk in Australia: A prospective study of uptake and psychological implications

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This prospective study is one of the first worldwide to examine uptake and implications of genetic testing for melanoma risk amongst individuals with a strong family history of melanoma. Families comprising three or more relatives with a confirmed melanoma diagnosis were ascertained via the Westmead Institute for Cancer Research/University of Sydney centre of the Genetic Epidemiology of Melanoma Study. Eligibility criteria included identification of a family-specific mutation in the *CDKN2A* gene via the research protocol, and no previous genetic testing for clinical purposes. A series of mailed, self-report questionnaires were used to collect data at: notification of genetic test availability (January 2005), and two weeks and 12 months after receipt of genetic test results (for 'test takers'), or 12 months after notification (for 'test decliners'). One-hundred twenty-one eligible individuals (48% male) returned baseline questionnaires (response rate of 72%). At baseline, mean psychological distress scores were relatively low. Factors associated with distress included: personal history of melanoma ( $OR=3.37$ ,  $p=0.03$ ), perceiving greater family-related consequences of melanoma ( $OR=2.52$ ,  $p<0.0001$ ), and displaying a tendency to monitor for risk-relevant information ( $OR=3.12$ ,  $p=0.01$ ). So far, 25 participants

have undergone genetic testing, with 75% of those who have received results identified as mutation carriers. At baseline, test takers reported significantly higher levels of distress compared to decliners ( $Z=-2.27$ ,  $p=0.02$ ). Carriers reported significantly reduced depression scores two weeks after receipt of a positive result ( $Z=-2.25$ ,  $p=0.02$ ). Distress was relatively uncommon in this familial melanoma cohort, even after receipt of positive genetic test results.

#### EPL4.1

#### Antenatal screening for fetal abnormality - ensuring informed choice for parents

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Antenatal screening for fetal abnormalities is available in many European countries. Previous research has identified that parental choice regarding whether to accept screening is limited by lack of information and the attitude of health professionals that the screening is 'routine'. The aim of this study was to identify the ways in which prospective parents can be supported to make informed decisions about antenatal screening.

In Phase 1, focus groups were held in 5 UK regions with prospective parents (pregnant women and partners) and professionals offering screening. Transcribed data were analysed using a grounded theory approach. In Phase 2, the focus group data were used to develop questionnaires. These were completed by prospective parents and relevant health professionals. Data were analysed using descriptive statistics and cross tabulations.

Findings indicate that parents feel overloaded with information at the start of the pregnancy about a range of topics; this restricts the attention paid to the screening decision. Some midwives were anxious when parents declined screening, due to fear of litigation. Appropriate information is frequently unavailable to those whose first language is not English or who are illiterate. Experience of the personal lives of people with disabilities influenced parents and midwives in their attitudes to screening. Parents were keen to have additional resources in this area. Resources focussed on the lives of affected persons and a decision-making tool for parents may help to ensure informed choice in antenatal screening. This information needs to be available in a comprehensible form for all parents.

#### EPL4.2

#### The role of attitudinal ambivalence towards Down's syndrome in prenatal testing decisions

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There is a growing consensus that decisions about prenatal testing should reflect the individual's attitudes and values: but what if the individual's attitudes are ambivalent? This study investigated the potential relationships between attitudinal ambivalence towards Down's syndrome and testing and termination decisions.

Data was collected from women attending an antenatal clinic in the UK. Using a questionnaire distributed at the booking appointment, attitudes towards using testing and termination for Down's syndrome were collected along with attitudes and attitudinal ambivalence towards Down's syndrome itself. Screening uptake was obtained from patient records. Overall, attitudes and attitudinal ambivalence towards Down's syndrome were not good predictors of screening behaviour and women with high levels of ambivalence were not less likely to use prenatal screening. However, women who were unsure about amniocentesis and termination were more likely to hold ambivalent attitudes towards Down's syndrome than were women who gave a definite yes/no response. In particular, a higher level of ambivalence about the effect of a child with Down's syndrome on parental quality of life predicted a 'don't know' response. Ambivalence correlated positively with higher levels of religiosity and was associated with a desire to make decisions in partnership with a significant other.

Psychological research shows that ambivalent individuals are more likely to be influenced by factors external to their own attitudes, such as the perceived opinions of others. The findings from this study suggest that holding ambivalent attitudes towards Down's syndrome may

have implications for the facilitation of 'autonomous' informed choice in the testing context.

#### EPL4.3

#### 'Balance' is in the eye of the beholder: perceptions of balanced information to support informed choices via AnSWeR (Antenatal Screening Web Resource)

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Objectives: AnSWeR (Antenatal Screening Web Resource), designed to support informed choices in prenatal testing, aims to provide balanced information about disability from the perspective of disabled people and their families. Following our independent evaluation of AnSWeR, this paper presents participants' evaluation of AnSWeR in terms of providing balanced information. Setting: U.K. Method: Eight focus groups with health professionals, participants from the general population, and parents with personal experience of the conditions. Questionnaires were completed by parents of newborns, people with spina-bifida or cystic fibrosis, and fifteen professionals with special expertise in this area. Findings: Information about experiences of living with the tested-for conditions and terminating affected pregnancies was considered important to support informed decisions. However, participants differed in their perceptions of whether the information about the tested-for conditions was balanced. For example, participants believed that photographs of people with the tested-for conditions introduced biases - both positive and negative. Within the context of supporting informed choice, participants also talked about the significance of providing information about women's experiences of terminating affected pregnancies. Conclusion: This study highlights the difficulty of designing 'balanced' information about tested-for conditions and a lack of methodology for doing so. We conclude that AnSWeR provides a counterbalance to other websites that focus on more medical aspects of disability. Its aim to provide 'balanced' information would be aided by increasing the number and range of case studies available on the website, for both family members and individuals living with the tested-for conditions, and women terminating affected pregnancies.

#### EPL4.4

#### Testing times, challenging choices; women, prenatal testing and genetic counselling

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Although prenatal testing is increasingly considered to be a 'routine' part of many women's pregnancy experience it is unclear how women experience this process or whether they are making informed decisions to participate. Genetic counselling exists to support women at this time but there is a dearth of research exploring either prenatal genetic counselling process or women's experiences. This research aimed to:-

- explore the experiences of women who received an increased result from a prenatal screening test, attended genetic counselling and made a decision about diagnostic prenatal testing

- examine, in detail, the process of prenatal genetic counselling

Women attending genetic counselling following an increased risk result from a screening test were invited to participate. Two data sets were obtained:-

1. 21 genetic counselling sessions were audiotaped, transcribed and analysed using content and discourse analysis (Data set 1)

2. 15 semi-structured follow up interviews with women from Data set 1 were audiotaped, transcribed and analysed thematically (Data set 2)

Rigorous qualitative research methodologies produced rich insights into counselling process and evocative accounts of women's experiences. Most women reported high levels of distress and decisional conflict and many women did not make an informed choice to participate in either screening or diagnostic testing. Research findings and theoretical literature are used to demonstrate that facilitating informed choices is an ethically appropriate model for prenatal genetic counselling practice. A contemporary model for practice, specifically addressing women's distress and actively encouraging clients to deliberate fully, is proposed and critiqued.

#### EPL4.5

#### „It's something I need to consider“: women's decisions about population carrier screening for fragile X syndrome

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Population carrier screening for fragile X syndrome (FXS) identifies carriers, and provides information for reproductive decision making. Few studies have explored women's decisions when offered carrier testing for FXS through a population screening program. This study is an in-depth exploration of factors that influenced women's choice regarding the carrier test.

In Victoria, Australia a pilot study was conducted offering carrier screening for FXS to women in a pre-conception setting. Women completed a questionnaire, were offered screening, and completed a second questionnaire one month later or after receiving their result. A selection of women then participated in follow-up interviews.

318 women participated in the study and 20% of women chose testing revealing one pre-mutation carrier and three grey zone results. 31 women were interviewed: 13 who chose to be tested, including three with positive results (two grey-zones, one pre-mutation); and 18 who chose not to be tested.

Factors that influenced test choice included: the woman's perception of the benefits of screening, her life stage and whether she had prior experience with health related issues. No women who were tested regretted their decision and it was clear that providing women with time aided the decision making process. Overall women were supportive of population carrier screening for FXS in a pre-conception setting.

This is the first attitudinal study to include women who declined screening as well as those who accepted. These results provide valuable insight into factors that influenced women's decisions regarding testing and will help inform future development of carrier screening programs.

#### EPL5.1

#### Verification of consumers' experiences and perceptions of genetic discrimination and its impact on utilisation of genetic testing

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A major component of the Australian Genetic Discrimination Project 2002-5 was to undertake a systematic process of verification of consumer accounts of alleged genetic discrimination, defined as the differential treatment of an asymptomatic person on the basis of their real or assumed genotype or genetic characteristics. Asymptomatic individuals reporting incidents in a survey conducted through Australian clinical genetics services 1998-2003 were recruited for the subsequent verification process. Others were recruited through genetics support groups and referrals from clinical genetics professionals. Verification of alleged incidents of genetic discrimination was determined, with consent, through interview, document analysis and, where appropriate, direct contact with the third party involved. Reported incidents of negative treatment in life insurance, employment, and health service domains met criteria for verification in 27/99 instances. Verification was possible in 14 cases (7 breast and ovarian cancer; 3 HNPCC; 3 Huntington disease and one each of hereditary haemochromatosis and polycystic kidney disease). All involved life insurance products. Issues included fear of genetic discrimination that impacted upon uptake and access to genetic testing for relatives; overly broad exclusion clauses; inability to increase policy amount; denial of insurance; coercion to access genetic test results and lack of recognition of prophylactic and screening strategies in underwriting decisions. In the course of verification, the decision-making process underpinning the life insurance underwriting was elucidated and reversal of adverse decisions following challenges to the company or provision of expert clinical genetics advice was confirmed. Verification is a potentially fruitful but a complex and challenging process.

**EPL5.2****The Multiplex Initiative: a study to determine who seeks free multiplex genetic susceptibility testing among a healthy population of American adults**

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Genetic tests designed to provide individuals estimates of their disease risk are now commercially available. In theory, this information can be used to guide risk reduction behaviors. Currently, little is known about who might avail themselves of such testing and how they would interpret their test results. To understand the impact of such testing, we developed a prototype genetic test including 15 polymorphisms associated with increased risk for eight common conditions. Healthy adults ages 24-40 receiving care at a large managed-care organization are offered testing via Web-based information modules (<http://multiplex.nih.gov>). Individuals opting for testing receive test results by mail, with telephone follow-up from a research educator; they are contacted three months after receiving test results for a final telephone survey. Our study design enables us to evaluate approaches that facilitate decision making about genetic tests, assess methods for communicating test results, and explore whether health system factors influence health outcomes. Baseline surveys have been completed on ~1600 individuals. For most, this is their first experience with clinical research. The majority of those completing the baseline survey are high school graduates, married, self-report being in excellent or good health, and are relatively familiar with their family's health history. So far, 472 individuals visited the Web site to consider testing; 272 have decided to undergo testing. Factors regarding reviewing information about testing on the Web and ultimate uptake of testing will be presented. Early results are already providing insight regarding how healthy individuals might respond to genetic susceptibility testing.

**EPL5.3****Willingness for blood donation for genomic studies: a comparative study between scientists and the public**

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Objectives: To compare attitudes between scientists and the public in Japan towards human genomic research and to seek new approaches for better science communication, we conducted questionnaire surveys and focused group interviews.

Methods: Self-reporting questionnaires were sent by post to 4,257 scientists regarding molecular biology and human genetics/genomics via two academic societies in Japan. They were asked about their value and risk cognition towards genome sciences, social norms for accountability and responsibility. We compared these results with the data we obtained from 2,171 citizens in 2005.

Results: A total of 1,494 completed our questionnaire (586 men and 157 women). The total response rate was 35.1%. Both groups support genome sciences very positively. The main difference was observed on willingness of blood donation for genomic research. 74.8 % of the scientist group responded that they were willing to donate for genomic research regarding healthcare, while 39.3% of the citizen group did. Regarding the conditions for donation, the scientist group responded that they would donate their blood when they could agree the research purpose and significance (87.3%). On the other hands, most of the citizen group responded that they would donate if researchers disclosed personal results (78.5%). From the FGI data to students, their understandings of "breach of personal information" and "disclosure of personal analyzed data" were obscure and were influenced by the media which criticized science. Discussion: We need to start discussions on the process of disclosure of personal analyzed data to each research participant for future human genomic research.

**EPL5.4****Privatising susceptibility to disease: the need for regulation?**

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As genetic technologies continue to reach new areas of healthcare, there are concerns whether genetic services might be compromised by consumer-oriented practices, especially promoted via TV or the internet. The marketing of genetic testing as capable of shaping lifestyles through the forecasting of future health may promise consumers more than the science can deliver, emphasising the supposed 'benefits of knowing' while downplaying the potential hazards. From the professionals' point of view, the marketing of commercial genetic services may misrepresent the complexity and uncertainty of genetic risk, obscuring reality with the fog of marketing. To illustrate our point, we consider a recent reality-based TV programme, broadcast in the UK, that documented the experiences of four 'celebrities' who consented to testing in a private London-based practice. Our analysis of the programme's transcript will show how interactions with the clients departed from established protocols of professional practice in several ways: overestimating clinical validity, underestimating the clinical uncertainty and the possibility of problems from test results, and practicing inappropriately directive ('promotional') counselling. How should the genetics services community in Europe respond to such developments? Our findings emphasise the already well recognised need for the development of regulatory and legislative approaches to monitor and/or prevent such practices. We consider several strategies for establishing standards of professional practice. One important concern is to ensure that too heavy-handed an approach does not inhibit the appropriate development of genetic services offered in good faith by non-specialist health professionals.

**EPL5.5****Gene patents and diagnostics: numbers don't count**

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Gene patenting is allegedly abundant and hampering access to genetic testing. This was given proof when Myriad Genetics exerted its exclusive rights to BRCA1/2. The case highlighted problems of patenting and licensing of genes and methods for genetic diagnosis, and laboratories' ignorance to patents, infringement, and risk for being litigated. In practice, laboratories usually lack legal expertise to engage in patent analysis and licensing negotiations. Patent abundance could create 'patent thickets', e.g. when many patents owned by several patent owners relate to a single test, and complicate licensing.

We aim at elucidating the patent situation in genetic diagnostics. Detailed patent landscape analyses of triplet repeat expansion diseases - Fragile X, Huntington disease, spinocerebellar ataxia, Friedreich ataxia, myotonic dystrophy, Kennedy disease - show patents' validity in time and territory, scope of protection, and, where possible, licensing mode. Both in Europe and US, disease-specific patents are granted, and applications under examination. Moreover, the patent situation of these diseases is complicated by 'generic' patents relating to their shared etiology. Broad in scope, these patents could also be overlooked in disease-specific patent searches because of their non-disease-specific nature.

In Europe gene patenting seems less abundant than proclaimed, in contrast to the US where patent thickets, and overlapping patent scope, are observed. Gene patent families not always have a European equivalent, or the European patent has lapsed in most, or all, countries. Despite low gene patent numbers in Europe as observed in this study, caution should remain towards broad patents and potential restrictive licensing conditions.

**EPL6.1****Psychosocial Impact of X-linked Carrier Status: Experiences of Female Adrenoleukodystrophy Carriers**

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We report the results of a qualitative study of the psychosocial impact of adrenoleukodystrophy (ALD) on female carriers. ALD is an X-linked metabolic disorder with extreme variability in phenotype, including the risk of symptoms in female heterozygotes. Carrier testing based on biochemical assay is unreliable, but accurate mutation based testing has been available in the UK since 1999.

Eighteen women with a confirmed ALD gene mutation were interviewed, and the transcripts analysed using the constant comparison method. The impact of the test result often correlated with the timing of the test, with less impact described by those who had requested testing in the mid teens, before reproduction, and more traumatic responses recalled by women who had testing at the same time as other emotional events, such as pregnancy. For the majority of women, carrier status had an ongoing impact, including feeling different and frequent intrusive thoughts. Women who had female relatives with symptoms, but were asymptomatic themselves, expressed anxiety about the risk of symptoms developing, against a perceived lack of medical understanding and treatment options.

These findings add to the broader literature on psychosocial impact of X-linked carrier status, and contribute new evidence that the implications for female carriers extend beyond reproduction. Genetic counselling should include discussion of the potential impact on self-image as well as living with the uncertainty of late onset manifestations in an increasing number of X-linked conditions where this is an issue.

#### EPL6.2

#### The marital relationship and psychological wellbeing in patients with Myotonic Dystrophy

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**Introduction:** Myotonic dystrophy (MD) is characterised by progressive muscular weakness and myotonia. Other organs are involved as well, including the brain, implying, among others, mental slowness and lack of initiative, and causing problems in daily life both for patients and their spouses. Some couples seem to deal with these problems satisfactorily while others experience great trouble.

**Objectives:** to describe the relationship of severity of MD, marital satisfaction and psychological well-being in MD-patients and their partners.

**Methods:** 69 MD-couples were interviewed regarding the influence of MD on their marital relationship, and they filled out questionnaires on severity of MD, anxiety and depression (HADS), hopelessness (BHS), and general psychological health (GHQ-12).

**Results:** For patients, the need for help was associated with a worse view on the future, a worse general well-being, more anxiety and more depression. For their partners, a lack of initiative was associated with a worse general well-being and more anxiety. Marital satisfaction was associated with a better view on the future for patients, and a better general well being, less anxiety and less depression in partners. It is remarkable that no less than 40 % of the patients and, particularly, female partners had BHS scores suggestive of clinically relevant depression.

**Conclusion:** Severity of MD places a heavy burden on patients and, especially, on female partners. Marital satisfaction is a strong predictor of a better well-being, both for patients and partners, but more so for the latter.

#### EPL6.3

#### Living with NF1: The perspectives of young people

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Neurofibromatosis type 1 (NF1) is a genetic condition of variable phenotype and progression, with several features (including learning difficulties and cosmetic effects) which may occur during childhood and adolescence. Previous psychosocial research on the effects on young people has largely been based on reports from parents or retrospective reports of affected adults on their childhoods.

This qualitative, exploratory study was conducted by two research-

ers and involved semi-structured interviews with fourteen adolescents (eight males and six females) aged 16-20 years. Analysis of the interview transcripts was performed using the constant comparative methodology.

Overall, both male and female participants reported that they had coped well with being affected by NF1, and although some identified that they felt 'isolated' or 'different' to others, this had usually not prevented them from 'getting on' with life. Some of the older participants felt they had learned to cope with NF1 better over time, although male participants frequently commented upon uncertainties regarding future progression.

The main areas of importance to both male and female participants were the challenges of talking to others about NF1, impact on education, developing friendships, relationships with parents, reproductive issues and concerns about the future. Cosmetic aspects of NF1 and employment concerns appeared more important to female participants.

While further studies are required to confirm and expand these initial findings, this study adds insight into the experiences and views of affected young people.

#### EPL6.4

#### Quality of life in hypertrophic cardiomyopathy mutation carriers

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**Background:** Hypertrophic cardiomyopathy (HCM) is a common autosomal dominant heart disease associated with heart failure and sudden cardiac death (SCD). Quality of life (QoL) was found to be impaired in symptomatic HCM patients but has never been assessed in mutation carriers, with or without manifest HCM.

**Methods:** From approached HCM mutation carriers, 89% (n=212) completed a questionnaire. QoL was assessed using the Short Form 36 Health Survey (SF-36) and the Hospital Anxiety and Depression Scale (HADS) and compared with reported QoL-data of HCM patients and the general Dutch population. Demographic, clinical and illness perception related variables were evaluated as predictors of QoL.

**Results:** HCM carriers' QoL did not differ from the Dutch population. Carriers with HCM had lower QoL scores than carriers without HCM, but higher scores than previously reported in HCM patients. Surprisingly, carriers without HCM scored significantly better than the general population on several QoL subscales. Best predictors of impaired physical QoL were: having symptoms ( $\beta=5.4$ ,  $p=0.001$ ) and stronger belief in serious consequences ( $\beta=4.2$ ,  $p<0.001$ ); predictors of impaired mental QoL were: stronger emotional reactions ( $\beta=2.0$ ,  $p=0.032$ ) and higher perceived risk of symptoms ( $\beta=0.7$ ,  $p=0.034$ ). Important predictors of anxiety were: female gender ( $\beta=1.3$ ,  $p=0.008$ ) and stronger emotional reactions ( $\beta=1.2$ ,  $p=0.002$ ). Stronger belief in serious consequences ( $\beta=0.9$ ,  $p=0.004$ ) and perceived risk of SCD ( $\beta=0.3$ ,  $p=0.002$ ) predicted depression.

**Conclusions:** HCM carriers' QoL is comparable to QoL reported in the Dutch population. Presence of manifest HCM influences QoL in carriers, but other variables, mainly illness perception related, are stronger predictors of QoL.

#### EPL6.5

#### Developing a cancer genetic-specific measure of coping

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**Background:** Generic measures of coping fail to capture the process of undergoing a specific health process such as cancer genetic risk assessment. A coping matrix has been developed to provide greater specificity of measurement, by breaking the risk assessment process into a number of specific stressors, and identifying the coping efforts used in response to each stressor.

**Methods** The matrix measures 11 recognised stressors and 8 specific coping strategies for individuals undergoing cancer genetic risk assessment, identified through previous research. The matrix was piloted within a psychological questionnaire as part of a randomised trial of a coping intervention (CARIAD).

**Findings** Preliminary analyses revealed that 134 of the 139 respondents completed the matrix, with the current data reported from the first 50 participants. Of the three most frequently endorsed stressors, 60% of respondents were quite a bit/very worried about how family members would react if found to be at increased risk, and primarily made use of social support; 54% were quite a bit/very worried about how they would cope if found to be at increased risk and primarily coped through positive appraisal; and 48% were quite a bit/very worried about having to wait to find out their own risk and coped mainly through acceptance.

**Discussion** Participants reacted in different ways to different stressors, although emotion-focused strategies were the most common overall. The completion rates for the matrix and specificity of responses provided suggests this coping matrix may be an acceptable measurement tool. Further data collection and validation is ongoing.

## EMPG WORKSHOPS

### EW1.1

#### Talking about disability in prenatal genetic counselling sessions: identifying tensions and developing strategies

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To facilitate informed decision-making in prenatal genetic counselling it is essential that counselees and counsellors engage in a process that provides:

- a) relevant information concerning the nature of genetic conditions
- b) an opportunity for counselees to consider parenting a child with a disability

This workshop will provide an opportunity for participants to reflect on the sensitivities inherent in such discussions and participate in developing strategies for best practice in addressing these complex issues. Academic literature and an ethical analysis of the goals of prenatal genetic counselling will be presented to demonstrate that facilitation of informed decision-making is an ethically appropriate goal of prenatal genetic counselling. Data from an Australian research project and the results of an interactive workshop at the 2007 NSGC Convention will provide further evidence of the need for more effective communication about disability in prenatal genetic counselling and identify complexities and impediments involved in doing so. Preliminary suggestions for addressing these issues will be presented.

A small group format will then be used in which participants will discuss and reflect on their experiences of communicating about disability, identify specific tensions and concerns associated with such dialogue, and develop strategies to promote effective and ethically appropriate discourse between counselees and counsellors. Written outlines from the groups will be used to develop a preliminary summary and synthesis, with further participant discussion. It is anticipated that this workshop will provide valuable information that can be complemented by input from consumer groups and other stakeholders.

### EW2.1

#### Assessing Quality of Counselling in the Context of Genetic Testing Workshop

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The EuroGentest network is a 5 year collaboration between many European experts, the aim of which is to harmonise and standardise genetic testing in Europe. In Unit 3, counselling issues have been the focus. Recommendations have been made about the need for certain types of genetic testing (e.g. prenatal, predictive) to be accompanied by appropriate genetic counselling, as well as psychosocial support in some instances, to safeguard those being tested. However, if genetic healthcare is to be evaluated or audited, appropriate standards and measurable outcomes need to be defined.

Assessing the outcomes of genetic counselling has long been a challenge to those in the field. Many outcomes (e.g. altered sense of control, peace of mind, ability to plan for the future) are difficult to measure. In addition, the structure of health services differs greatly across European countries. The Expert Group of Unit 3 has devised an assessment tool, setting standards and potential measurable outcomes for genetic counselling. The standards address aspects of the service including access to peer support and continuing professional education, supervision of junior staff, waiting times, physical clinical environment and communication with counselees. A set of measures that can be applied across different systems of healthcare has been drafted.

In this workshop, we will present work already undertaken, but the main aim will be to engender discussion and to use the feedback and ideas generated to further develop a set of assessment tools that will be applicable to genetic counselling services in the European context.

### EW3.1

#### Workshop: Genetic Counseling and predictive testing: A dynamic perspective

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Genetic counseling aims to allow counselees to make informed decisions and act accordingly. In this workshop we wish to explore how genetic counseling and the procedure of predictive testing can benefit from both psychodynamic and family therapy theory and practice. First, psychodynamic theory and its application in genetic counseling will be clarified using four models: the developmental, object-relationship, the self-psychology and the drive model. Second the relevance of and application of structural family therapy theory will be described. Third, elaboration of two clinical cases will show the usefulness of these theories. Finally, we will explore the requirements of training and education, in both psychodynamic and structural family theory and practice, in genetic counseling. Participants are invited to bring their own cases.

### EW4.1

#### Analysing the social dimensions of coping in families: a workshop on presenting the results in clinical practice

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Coping is commonly viewed as an individual stress response, but it has also a social dimension. How do people experience stress in social groups, like families? How do they engage together in actions to deal with a stressor, like a genetic disease? In clinical practice, obtaining a family history is an essential skill for genetic counsellors. It provides a basis for making a diagnosis, determining risk, and assessing the needs for patient education and psychosocial support. Valuable psychosocial information can be obtained in parallel with medical-genetic information. The family pedigree however does not show data on social interactions within the family, although 'the process [of gathering family data] frequently induces a thoughtful frame of mind that is both inward- and outward-looking regarding family members and family dynamics'. (Weil, Psychosocial genetic counseling, 2000).

In this workshop, we start with an introduction of a conceptual model on social dimensions of coping, illustrated by data from a family questionnaire we used in a study on coping strategies. Next, we present some tools by which the information about social interactions within a family visually and conceptually can be organized. These tools can be helpful in analysing the dynamics of a family: kin and nonkin relationships, communication patterns, social roles and messages. In this way

insight is gained into the processes within the nuclear and blended family and the history of the extended family. This insight can be useful in getting a process of change going in the way counselees cope with the genetic information.

## EMPG POSTERS

### EP01. Reproductive issues in genetics

#### EP01.01

##### How can prenatal genetic counseling be conducted in a country where abortion is not considered as women's right?

**C. Tamura;**

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Abortion in Japan is primarily illegal with the exception of special reasons, such as maternal health threats, financial difficulties, etc. Fetal problems are not included in these exceptions. If women want abortion, they have to ask permission from doctors and partners. When prenatal diagnosis revealed fetal problems, doctors use a financial reason as an excuse to allow abortion. In this way, prenatal diagnosis and selective abortion is available. About one percent of all pregnant women undergo amniocentesis. Professional societies established guidelines for prenatal diagnosis, and state that it can be conducted only to test childhood-onset severe conditions. If women want prenatal diagnosis, doctors, often clinical geneticists, have to make ethically sound judgment, and will tell women whether or not they can undergo testing. Although genetics societies have certified some non-MD genetic counselors recently, genetic counseling in Japan is supposed to be provided by clinical geneticists, as they have done for over thirty years. And, clinical geneticists are regarded as authorities who make ethical judgment on prenatal diagnosis and selective abortion. Thus, genetic counseling in Japan seems to be a place where clients need to make an inquiry to ask permission to undergo prenatal diagnosis, and geneticists sometimes try to convince women not to undergo testing, or sometimes give clients permission to do it.

Appropriate prenatal genetic counseling approach in Japan should be considered, but, it is difficult because those who are regarded as genetic counseling providers believe that their current practice is an appropriate way of genetic counseling in Japan.

#### EP01.02

##### PGD for BRCA - a novel clinical experience

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The option of offering PGD for BRCA1/2 to carriers who are of reproductive age was recently discussed in the literature. This raises medical, psychological and ethical dilemmas. Our cancer-genetic counseling team started recently to discuss this option with young carriers. Six women, 4 of them carrying a mutation in BRCA1 and 2 in BRCA2, applied for further genetic and reproductive counseling in our PGD clinic. Four of these carriers were healthy but had at least one 1<sup>st</sup> degree relative with breast cancer (BC), and 2 were 4 years post BC diagnosis. All women needed IVF because of coexisting infertility. After counseling, 4 women declined the option (including the two BC survivors who eventually conceived naturally) and 2 underwent the PGD procedure. One of them conceived in her first treatment attempt. In this case, 8 embryos with 7 or more cells were biopsied. The blastomeres were analysed, using PCR for the BRCA2-6174delT mutation together with linked polymorphic microsatellites. Four embryos were conclusively diagnosed as BRCA2 wt, 2 of these were transferred, leading to a twin pregnancy. Because the woman would not have terminated a pregnancy in case of misdiagnosis, amniocentesis was not performed. Her request to confirm the PGD in her newborns after birth raises additional legal and ethical dilemma of testing minors for adult onset disease.

#### EP01.03

##### Hereditary breast/ovarian cancer predisposition and reproductive decision-making

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**Purpose:** Mutations in the *BRCA1* and *BRCA2* genes predispose individuals to breast, ovarian, prostate and other cancers, generally from the mid-third decade onwards. Penetrance is not complete, and options for substantial risk reduction are available. A subset of people cite reproductive decision-making as a motivation for undergoing predictive testing, but very little is known about how *BRCA* status impacts on these decisions. With the recent (2006) decision by the HFEA to grant licences for pre-implantation genetic diagnosis for late onset cancer predisposition, this ongoing study exploring reproductive behaviour and attitudes to prenatal testing and PGD is timely.

**Method:** A qualitative approach using semi-structured, in depth interviews, analysed using interpretative phenomenological analysis. We aim to recruit up to 40 women and men who have had a positive *BRCA1/2* predictive genetic test during the preceding 5 years, between the ages of 18 and 45, who did not have children at the time they were tested.

**Results:** Data will be presented from analysis of interviews completed to date. Preliminary analysis indicates that *BRCA* status does affect reproductive decision making in a variety of ways, including timing of having children and number of children planned. *BRCA* status is also taken into account in partner-relationship building. Prenatal testing, either pre- or post- implantation would not be considered by interviewees personally, although they would not object to use by other *BRCA* carriers.

#### EP01.04

##### Attitudes of young women with cystic fibrosis to pregnancy and motherhood

**S. J. Kenrick<sup>1</sup>, E. Chapman<sup>2</sup>;**

<sup>1</sup>Addenbrooke's Hospital, Cambridge, United Kingdom, <sup>2</sup>Papworth Hospital, Cambridge, United Kingdom.

Over the past two decades, improvements in treatment have resulted in most patients with cystic fibrosis (CF) reaching adulthood. Consequently, more affected individuals are becoming parents. Pregnancy is not considered dangerous for women with mild disease and good lung function, but health and survival may be compromised by pancreatic insufficiency and poor lung function. Clearly, careful counselling is required to discuss the risks of having an affected child, health risks of pregnancy as well as how to cope with illness while raising a family. The purpose of this pilot study was to explore psychosocial issues concerning pregnancy and motherhood among young women with cystic fibrosis, in order to inform reproductive counselling. Semi-structured interviews were conducted for five women with CF, aged between 20 and 25, and transcripts subjected to qualitative, thematic analysis. Emergent themes highlight a wide variety of issues for these women centred around; desire for a family, health risks, relationships and communication with others, difficulties in motherhood and the prospect of a child with CF. The results show that young women with CF may have varying attitudes towards pregnancy and motherhood, depending in part on their current health, their upbringing, professional opinion and social support available. Participants with a strong desire to have children viewed this as a natural progression and described strategies to cope with potential health problems. The study also highlights a number of measures that may help in counselling these women, including access to the experience of mothers with CF.

#### EP01.05

##### Reproductive decision making in CF carrier couples; an explorative study in couples with an affected child

**R. Jassi, W. H. Beckett, T. Roberts, M. McAllister, D. Scotcher;**

Regional Genetics Service and Medical Genetics Research Group, CMMC NHS Trust and University of Manchester, Manchester, United Kingdom.

This study is part of wider qualitative research project exploring reproductive decision making in cystic fibrosis (CF) carrier couples. Here we describe the personal experience of 19 participants living with the condition because they had a child affected by CF. Men and women in each couple were interviewed separately by different researchers in

their own home.

It emerged from the analysis that different approaches were taken by participating parents before they reached a reproductive decision, and while several factors influenced this, the first diagnosis of CF in the family had a particularly significant influence. Parents in this study appeared to have undergone an adaptation process during which they overcame the initial shock of a CF diagnosis in their child, developed a more positive outlook based largely on their own experiences of CF, and then felt able to address further reproductive decisions. However, following adaptation, some differences between the sexes emerged. Women frequently acknowledged that the decisions remained fluid and dynamic, whereas men tended to portray a more rigid standpoint once a particular decision had been reached. Furthermore, men and women often felt that they had inherently different roles in reproductive decision making, with the ultimate decision resting with the woman. In most cases, couples felt that the decision was made together, without significant external influence.

#### EP01.06

##### **Uptake of genetic counselling and prenatal diagnostic services for CF in Ireland**

**D. M. Lambert<sup>1,2</sup>, M. Morgan<sup>1</sup>, S. Lynch<sup>2</sup>**

<sup>1</sup>Children's University Hospital, Dublin, Ireland, <sup>2</sup>National Centre for Medical Genetics, Dublin, Ireland.

Cystic fibrosis (CF) is a common recessive condition in Ireland with 1 in 19 carriers and 1 in 1440 affected. From January 2004 to July 2007, 76 patients were sent appointments for genetic counseling for CF: 23 (30%) were routine referrals from the CF clinic for a new CF family; and 53 (70%) were GP referrals for positive family history.

For GP referrals the risk of being a CF carrier was: CF affected (3.7%), known carrier (20.7%), 2/3 risk (22.6%), 1/2 risk (41.5%), 1/4 risk (7.5%), population risk (1.9%). 66% had a partner while 34% wanted to discuss carrier status before finding a partner. Those at higher risk of being a carrier were more likely to wait until they had a partner before being referred. Five of 53 (9%) were found to be at a 1 in 4 risk of CF. Of 25 families with a child with CF, 21 of the 23 referred attended the genetics appointment. Of the 30 families at 1 in 4 risk of CF, 15 (50%) remained childless since. For 26 of 30 couples a molecular prenatal diagnosis was available, with 12 having a pregnancy and 5 (42%) a prenatal test. 4 indicated interest in pre-implantation genetic diagnosis (PGD). 6 families (20%) had members seen for cascade screening although the offer was made to all. The uptake for prenatal diagnosis and screening of family members is lower than other European countries, perhaps reflecting the illegality of termination and PGD in Ireland.

#### EP01.07

##### **Reproductive decision making in CF carrier couples; an explorative study in couples without an affected child**

**J. Myring<sup>1</sup>, R. Sayers<sup>2</sup>, T. Roberts<sup>3</sup>, D. Scotcher<sup>3</sup>, M. McAllister<sup>4,3</sup>**

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We report on part of a larger research project which uses qualitative methodology to explore the reproductive decision making process in CF carrier couples. 5 males and 7 females from CF carrier couples without an affected child were interviewed individually in their home, to explore both male and female perspectives of the reproductive decision making process.

Data analysis revealed that while couples may consider different scenarios, the decision making process remains largely unstructured. Personal experiences of CF were an important factor: Individuals had to weigh up their perception of the physical difficulties of CF, the fact that CF is not an intellectually disabling condition, and their attachment to someone with CF. Individuals may not fully engage with their risk of having a child with CF during the decision making process, which may lead them to be unprepared for bad news.

Men and women may play different roles within the reproductive decision making process. Men appeared to take on a supportive role, tending to agree with their partner. Women felt that the decision was mutual and intuitive, possibly due to this support from their partner. Both partners felt that ultimately, the decision was primarily the woman's;

influenced by the physical attachment to pregnancy and possibly their role as primary carer.

CF carrier couples who have a healthy child in their first at-risk pregnancy may feel that they have "used up their luck", which may lead to a decision to limit family size.

#### EP01.08

##### **Ancestry-based preconceptional CF and HbPs carrier screening in a multi-ethnic population: attitude and participation, psychological outcomes, reproductive intentions and satisfaction**

**P. Lakeman, A. M. C. Plass, L. Henneman, P. D. Bezemer, M. C. Cornel, L. P. ten Kate;**

*VU University Medical Center, Amsterdam, The Netherlands.*

**Objective:** to investigate attitude and participation, psychological outcomes, reproductive intentions and satisfaction in ancestry-based preconceptional carrier screening for cystic fibrosis (CF) and hemoglobinopathies (HbPs).

**Methods:** 9,453 individuals were offered carrier-testing, which was conditional on survey-participation. Eligible for test-participation were invitees who were planning a pregnancy with their partner. Both partners' ancestry determined eligibility for the CF and/or HbP-test(s). Data were gathered with structured questionnaires, one of which was based on the Theory of Planned Behaviour, among 418 invitees of whom 247 refrained from testing and 171 intended to participate in the testing, but of whom 143 actually did. Non-Western participants (n= 46) were under-represented.

**Results:** All survey-participants, Western and non-Western, had a positive attitude towards test-participation. Among those who refrained from test-participation, 68% would participate in this kind of screening in the future if it became possible. Time and effort needed for participation were important declining factors. The majority reported no predominant feelings of stigmatization. More non-Western (23%) than Western participants (10%) thought that there would be discrimination against carriers. In general, the test-participants reported low levels of anxiety, intended to draw reproductive decisions from test-results, were satisfied and none of them regretted participation.

**Conclusion:** Ancestry-based preconceptional CF and HbPs carrier screening was evaluated as positive and desirable among Western and non-Western participants. No major adverse psychological outcomes were reported. The effort and time needed for participation was an important reason for declining participation, which might be overcome by facilitating access to the screening.

#### EP01.09

##### **Recruiting for reproductive choices**

**S. Karner<sup>1</sup>, B. Wieser<sup>1</sup>, W. Berger<sup>2</sup>**

<sup>1</sup>IFZ, Graz, Austria, <sup>2</sup>IFF, Klagenfurt, Austria.

Prenatal testing has become a routine practice of antenatal care. According to international standards informed consent is a requirement prior to testing. However, due to the introduction of non-invasive screening (nuchal translucency measurement and the combined maternal blood test), this arrangement is undermined in some important ways. There is a tendency that counselling starts not before the first screening, but only if the results are conspicuous.

In Austria antenatal care is organised in a comprehensive programme that is mandatory for all pregnant women. The aim of this programme is to secure the wellbeing of both the mother and her child. We argue that because of the contextualisation of non-invasive screening into the routine antenatal care it is difficult for pregnant women to distinguish between mandatory and elective examinations of antenatal care. This is especially relevant for the quality of informed consent that can be achieved in prenatal screening and subsequent diagnostic testing of the foetus.

The presented case addresses recruiting processes for prenatal testing and subsequent reproductive choices. We will present data which show how non-invasive screening has significantly changed prenatal testing in Austria. From 2002 on there is a sharp increase in diagnosed cases per tested pregnant woman. The current abortion rate of Down's syndrome foetuses is about 94%. The remaining 6% include those who screened false-negative, decided against screening or testing, and those who decided not to terminate the pregnancy.

**EP01.10****Association of CoL1A1 Gene alleles with the development of symphysis joints pathology in pregnant women in the Kazakh population****S. K. Kyzdarbaeva L.I.:**

NCAGP, Almaty, Kazakhstan.

Pregnancy has a negative influence on the skeleton and the development of osteoporosis. Patients with symptoms of symphysiolysis are seen in the symphysiopathias clinic.

**Objective** To investigate the distribution of TG polymorphisms in the CoLI A1 gene with development symphysis joints pathology of Kazakh population.

**Subjects** DNK 42 healthy pregnant females (the control group) and 30 pregnant with symphysiopathias (patient group).

**Results.** The GG genotype was present in 64,2% of Healthy pregnant women and in 36,6%, of the patient group ( $p<0,05$ ). The TT genotype was 3 times more prevalent in controls than in patients (16,6 % and 4,7 % respectively). Prevalence of the T allele in the control group was 20,2 %, but it was only - 40,0  $\pm$  4,8 % in the patient group ( $p<0,05$ ).

**Conclusion.** This study demonstrated that the T- allele of the CoL1A1 gene may be a predictor of susceptibility for symphysiopathias.

**EP01.11****The study of reproductive behavior in exposed populations****P. W. Izhevskij:**

State Research Center - Institute of Biophysics, Moscow, Russian Federation.

The aim of this research was the study of possible unfavorable influence the radiation factor on the populations by means:

- analysis of the radiation doses absorbed by population;
- determination of pre- and post-accidental rate of unfavorable pregnancy outcomes (UPO) in exposed populations.

The materials were collected in 226 populations of Belorussia and Russia. The given districts have different levels of  $^{137}\text{Cs}$  surface contamination: up to  $111 \times 10^4$  - to  $148 \times 10^4 \text{ Bk/m}^2$ . Doses accumulated since 1986 to 1992 was rated for a person who was continuously resident in studied populated areas.

The materials were collected retrospectively by interview by specially form. Women (2233) from a random sample, who were continuously resident, both 1208 of them lived in town and 1025 lived in the country. All the pregnancy outcomes of questioned women at reproductive age in the two temporary intervals: preaccidental (1980 - 85) and postaccidental (1986 - 92).

In the postaccidental period UPO rate reliably increased and the increase rate was much more in the united population. The data obtained don't allow us to isolate reliably the radiation-induced component in postaccidental reproductive behavior of the studied populations.

In populations which were exposed to ionizing radiation, in postaccidental period were noted the decrease of the live-birth rate and increase of the medical abortion's rate, that can be caused the stress situation and the decrease of standard of living.

**EP02. Genetic risk and testing: impact on****men****EP02.1****PSA Screening: A comparison of attitudes and behaviour in US and UK men with a family history of prostate cancer****M. Watson<sup>1</sup>, R. McCurdy<sup>2</sup>, J. Bloom<sup>3</sup>, C. Moynihan<sup>4</sup>, S. Stewart<sup>5</sup>:**

<sup>1</sup>Royal Marsden Hospital, Sutton, United Kingdom, <sup>2</sup>University of California Berkley, San Francisco, CA, United States, <sup>3</sup>University of California Berkeley, San Francisco, CA, United States, <sup>4</sup>Institute of Cancer Research, Sutton, United Kingdom, <sup>5</sup>University of California, San Francisco, CA, United States.

**Purpose:** This study compared PSA screening, attitudes and risk perceptions of US and UK men with a family history of prostate cancer.

**Methods:** 272 healthy white males [150 US/122 UK], aged 35 - 74 with a family history of prostate cancer. Multivariate logistic regression tested associations between screening behaviour and components of the health belief model, adjusted for socio-demographic and family history variables.

**Results:** College educated men and those with more cases of prostate cancer in the family were more likely to rate their prostate cancer risk as "greater than average". Having a family member with prostate can-

cer significantly affected PSA uptake [ $p<.01$ ] and perceived benefits to PSA screening [ $p<.01$ ]. Health anxiety was associated with prostate cancer worry and perceived risk [ $p<.05$ ]. Men were more likely to have a PSA test if older, married, reporting perceived benefits to PSA screening, and rating personal risk as greater. US men were more likely to have a PSA test and reported higher perceived risk of prostate cancer and perceived benefit of testing. The cost of PSA testing was a barrier [ $p<.05$ ](US only).

**Conclusions:** Findings support the view that family history is a determinant both of PSA uptake and perceptions of prostate cancer risk. This study found some differences between US and UK men. Variations are most likely associated with health system differences.

**EP03. Genetic risk and testing: carriers of x-linked conditions****EP03.1****Cardiac screening in Becker and Duchenne Muscular Dystrophy carriers known to the North West Genetic Family Register in Manchester, UK****P. D. Greene, G. Hall, H. Kingston, L. Kerzin-Storrar;**

Regional Genetics Service and Medical Genetics Research Group, CMMC NHS Trust and University of Manc, Manchester, United Kingdom.

We report patient responses and cardiac outcomes of 122 Duchene and 43 Becker Muscular Dystrophy carriers (aged between 20-77years) who were offered cardiology screening as part of their annual register contact. The Becker (BMD) and Duchenne Muscular Dystrophy (DMD) Register was set up in 1980 to foster long term contact with families and to offer genetic counselling, carrier testing and discussions around reproduction for women in families with muscular dystrophy.

The evidence of cardiac complications in dystrophinopathy carriers has been widely reported resulting in guidelines for cardiology screening from the 107<sup>th</sup> ENMC (European Neuromuscular Centre) International Workshop. In the light of these we wrote to confirmed / obligate carrier women informing them of their cardiomyopathy risk. So far, 95 DMD and 33 BMD carriers have requested referral for cardiac screening, the majority of whom were happy to be referred directly and only a few described shock or anxiety upon receiving their letter.

Dystrophinopathy is one of several X-linked conditions in which symptoms amongst carrier women are increasingly recognised. We will explore the role and responsibility of genetic registers in re-contacting women about unforeseen complications and the acceptability of this from the patient's perspective.

Cardiac screening amongst this cohort showed a much lower incidence of clinical problems than had been anticipated from previous studies.

**EP04. Genetic conditions: impact on significant others****EP04.1****Legacy for the living - A collaborative model of referral between state coroner's office and a clinical genetic service****R. M. Forbes<sup>1</sup>, R. Savarirayan<sup>1</sup>, M. Lynch<sup>2</sup>, N. Morgan<sup>3</sup>:**

<sup>1</sup>Genetic Health Services Victoria, Parkville, Victoria, Australia, <sup>2</sup>Victorian Institute of Forensic Medicine, Southbank , Victoria, Australia, <sup>3</sup>Victorian Institute of Forensic Medicine, Southbank, Victoria, Australia.

The Coronial Service Centre receives approximately 4000 reports of deaths each year. The majority of these reports are referred to the VIFM for autopsy to establish cause of death. During the course of a forensic investigation into the cause of death the pathologist may discover information of direct relevance to the health of first-degree family members. Traditionally exchange of information between forensic pathologist and families has been limited due to emphasis placed on the legal nature of the investigation.

We present a program that has been successfully established between the Family Liaison Coordinators at VIFM and GHSV. Families identified as being "at risk" of a genetic condition are given the opportunity to discuss findings of the autopsy. Implications for surviving family members is discussed and the availability of referral to GHSV, with family consent, is provided. Due to the complexity and specialised nature of the conditions, benefits of collaboration have been two-way

with resultant specialist care for the families.

An audit of conditions referred to GHSV, uptake by families, role of genetic counsellor at coronial and clinical service and outcomes over a 2 year period is discussed and presented as a model for future and extended collaboration between forensic and clinical medical genetics unit.

## EP05. Access to genetic services

### (challenges in Europe)

#### EP05.1

##### **Why don't Deaf People come for Genetic Counselling?**

*A. Middleton<sup>1</sup>, S. Emery<sup>2</sup>, G. Turner<sup>2</sup>, A. Clarke<sup>1</sup>, S. Sarangi<sup>3</sup>;*

<sup>1</sup>*Institute of Medical Genetics, School of Medicine, Cardiff University, Cardiff, United Kingdom, <sup>2</sup>Department of Languages & Intercultural Studies, School of Management & Languages, Heriot-Watt University, Edinburgh, United Kingdom,*

<sup>3</sup>*Health Communication Research Centre, School of English, Communication and Philosophy, Cardiff University, Cardiff, United Kingdom.*

Despite genetic deafness being one of the most common genetic conditions, deaf adults very rarely access genetic counselling, neither to discuss deafness nor other conditions that may be running through their family, e.g. cancer. Each genetics centre in the UK will have several thousand deaf sign language users within their catchment area; however, referrals are likely to be received for less than 5 deaf adults per year. There may be many complex reasons behind this - e.g. lack of information, assumptions about inheritance, mistaken beliefs of a link between present-day genetics services and eugenic practices of the past or fears about being told not to have children. This project aims to gather the views of various different groups of people with deafness, looking specifically at the above issues. 30 interviews have been conducted in sign language and qualitative analysis performed. Video clips of deaf people signing their views about genetics will be shared as well as results from a large ( $n = 1000$ ) quantitative study gathering attitudes. The research findings offer suggestions as to why currently genetics services are very rarely used by deaf patients in the UK. Once more is understood about this then steps can be taken to address access issues.

#### EP05.2

##### **Genetic Led Multidisciplinary Clinics As Communities of Practice**

*S. A. Watts;*

*Guy's Hospital, London, United Kingdom.*

Care for patients and their families with genetic disease has become increasingly complex in recent years due to a rapid rise in scientific knowledge, expanding technological innovation and demand for services, resulting in a need for multidisciplinary working. Although such working is well documented in general healthcare there is sparse literature within the specialty of clinical genetics. The results of an ethnographic study, which sought to examine the way in which patient care was organised and delivered in three multidisciplinary clinics in a Regional Genetic Centre in the UK, will be presented. Different models of healthcare were identified. However, data analysis using the theoretical framework of Communities of Practice (Wenger 1998), a theory of social learning and participation, suggests that functioning of the clinics is more closely related to the way in which clinicians establish a specific pattern of working together. The principal factor that contributes to the success of the clinics is that of coherence. The Genetic Counsellors play a crucial role to this coherence by acting as maintainers of the clinic. The results of this study contribute to our understanding of multidisciplinary working within clinical genetics and are important for the future planning and implementation of healthcare for patients with genetic disease.

#### EP05.3

##### **European Network of Genetic Nurses and Counsellors**

*C. Patch<sup>1</sup>, H. Skirton<sup>2</sup>;*

<sup>1</sup>*Guys and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>2</sup>University of Plymouth, Taunton, United Kingdom.*

While there are a growing number of non-medical genetic practitioners in clinical practice in Europe, there is no European-wide organisation dedicated to the needs of those professionals. In an environment

where the numbers of genetic tests are growing, the need to educate and develop non-medical practitioners who are competent in genetics is essential. Importantly, nurses and counsellors may also be working in situations with few colleagues, where peer support is hard to access. To address these needs, late in 2007, with the support of the ESHG, a new European Network of Genetic Nurses and Counsellors was initiated.

An invitation to join the network was sent to EuroGentest and ESHG members, requesting that they pass it on to any genetic nurses or counsellors known to them in their countries. Language is a difficulty and colleagues from several countries have offered to translate the information for colleagues who do not speak English. It is clear from the responses that professionals in many countries are trying to develop this new profession and are keen to use the network for support during this important period.

A questionnaire has been sent to establish the aims and focus of this network and the findings will be presented at an initial meeting of the network at the EMPAG/ESHG conference in Barcelona

As of February 2008, the network has 72 registered members from Netherlands, France, Spain, Portugal, Denmark, Sweden, Finland, Italy, Croatia, Bulgaria, UK, Czech Republic, Greece, Belgium, Cyprus, Ireland, Turkey, Israel, Switzerland, Poland and Iceland.

#### EP05.4

##### **The value of personal interaction: can a family history questionnaire replace a face-to-face family history gathering appointment with the genetic counsellor for parents of children with an undiagnosed genetic syndrome?**

*B. J. Dell<sup>1</sup>, G. Westwood<sup>2</sup>, C. Phelps<sup>3</sup>;*

<sup>1</sup>*School of Medicine, Cardiff, United Kingdom, <sup>2</sup>Wessex Clinical Genetics Service, Southampton, United Kingdom, <sup>3</sup>Institute of Medical Genetics, Cardiff, United Kingdom.*

Evidence suggests that parents often feel unprepared for their contact with Genetic Services (Skirton, 2006). Whilst family history questionnaires (FHQ) are increasingly used to gather data prior to attending a genetics clinic, such questionnaires have been found to raise anxiety (Phelps et al, 2006) and can act as a barrier to accessing services (Geer et al, 2001). This pilot study explored the impact of completing a specially developed FHQ upon subsequent perceptions of providing a family history face-to-face.

Seven parents completed the FHQ and an evaluation questionnaire. Results revealed that whilst the completion of the FHQ did not have any overall negative impact, it did have the potential to raise distress when asking for sensitive details such as miscarriages.

Four parents also completed telephone interviews to explore their perceptions of the FHQ and their appointment with the Genetic Counsellor. Thematic analysis revealed that parents encountered obstacles to the completion of the FHQ and that they appreciated their personal interaction with the Genetic Counsellor, which provided an opportunity for them to gain reassurance, to ask questions about the referral process and to clarify the relevance of the family history details.

The combination of both the FHQ and the Genetic Counsellor clinic was the preferred option, permitting the parents to be better prepared to utilise their contact with the genetic services.

## EP06. Lay beliefs and public understanding of genetics

#### EP06.1

##### **Using ethnography to optimise clinical practice in cancer genetics services**

*M. T. Saleh<sup>1,2</sup>, B. Meiser<sup>2</sup>, K. Tucker<sup>3</sup>, J. Kirk<sup>4</sup>, M. Eisenbruch<sup>5</sup>, K. Barlow-Stewart<sup>1</sup>;*

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This project explores, using ethnography, the cultural beliefs about inherited susceptibility to cancer amongst the Arabic-Australian Community.

A group of 15 individuals who identify themselves as Arabic-Australian were recruited through two Sydney familial cancer services. Ethnographic interviews explored themes such as beliefs about inherited illnesses including inherited susceptibility to cancer, cultural beliefs and practices when communicating within families about inherited cancers and the impact, if any, of attending a cancer genetics service on these beliefs and practices. Thematic analysis of interview summaries identified common outcomes, informing the development of an explanatory model for inherited susceptibility to cancer in this sample.

The cultural backgrounds of the 7 males and 8 females interviewed were Egyptian, Iraqi, Lebanese and Turkish, and ages ranged from 29 to 82 years (median age 54). Participants generally were highly acculturated as measured by their English language proficiency and had a good understanding of the genetic basis of the cancer in their family. There was little or no discrepancy between the individuals' beliefs about inherited cancer and the model of clinical practice utilised in the clinics attended.

People of an Arabic-Australian background are one of the fastest growing ethnic groups in Australia. It is therefore important to document their beliefs to ensure they are compatible with the current models of practice in cancer genetics clinics.

A better understanding and awareness of cultural beliefs and potential areas of disparity will guide optimal cancer genetics service provision. More research is needed to confirm reported beliefs of non-attending family members.

## EP06.2

### The public understanding of newborn screening

**B. Wieser;**

IFZ, Graz, Austria.

Neonatal screening for genetic disorders is a routine practice for many decades. In particular, screening for PKU has become a standard procedure. However, internationally the way by which neonatal screening is carried out varies notably. Especially for disorders where treatment options are not as straightforward as they are for PKU, screening may be offered, but only on voluntary basis. Austria has decided to expand its programme significantly in 2002. The introduction of tandem-mass-spectrometry plays a crucial role in this regard. With this technology it became possible to screen for 30 inherited disorders. All together the Austrian neonatal screening programme comprises as many as 23 conditions.

In this paper I will especially address the issue of the public understanding of newborn screening. I will do so by drawing on the Wilson and Jungner guidelines as developed for the WHO. Specifically I will address the 6<sup>th</sup> principle: "The test should be acceptable to the population" (cf. Dhondt 2007). Taking this requirement seriously this raises important issues of lay understanding of genetic disorders and the way in which an expansion of newborn screening can be perceived. The issue will be contextualised into the historical context in which the Austrian newborn screening programme has been initiated. I will also contrast the Austrian case from other European countries in order to point out differences of the way in which a public understanding of newborn screening can be facilitated.

## EP06.3

### Why do intenders not behave: exploring the gap between the intention to take part in preconceptional carrierscreening for cystic fibrosis and/or haemoglobinopathies and not doing so

**A. C. Plass<sup>1,2</sup>, P. Lakeman<sup>1</sup>, J. Wenink<sup>1,2</sup>, M. C. Cornel<sup>1,2</sup>;**

<sup>1</sup>VU University medical center, Amsterdam, The Netherlands, <sup>2</sup>EMGO-institute, Amsterdam, The Netherlands.

The aim of this study was to explore why couples who intended to participate in a preconceptional carrierscreening for cystic fibrosis and/or haemoglobinopathies (N=142), did not take part in the test after all (N=66), particularly beyond reasons like of lack of time.

All 66 couples were called up and asked whether they were willing to answer a few questions directly or some time later at their home about their non-participation in the test. All phone calls and face-to-face interviews were recorded and analyzed using the IPA-method.

41 couples were contactable. 13 couples no longer belonged to the research target group of couples that were planning a pregnancy in the future, and were excluded. 20 couples agreed to a telephone-interview and 5 others were interviewed at their home. The reason most

frequently mentioned for not taking part in the test after all was 'lack of time'. But, in asking about social influence it appeared that sometimes parents, other family members, friends or GPs had expressed their doubts about the couple's intention to take part in such a test.

Even though lack of time was the most frequently mentioned reason for not taking part in a preconception carrier-screening test after clearly having expressed the intention to do so, the negative input of the social environment might have played a major role in not carrying out one's intentions. Most couples were surprised by the negative reactions of their social environment and reevaluated test participation as being less important.

## EP06.4

### Attitudes towards thalassaemia carrier testing in saudi arabia

**H. Y. Alabdulwahed;**

Dhahran Health Center, Saudi Aramco, Dammam, Saudi Arabia.

Thalassaemia is a life limiting condition, characterised by severe anaemia which requires frequent blood transfusions. It is a global health problem, and is prevalent in countries with a history of malaria outbreak. Thalassaemia is a recessively inherited condition, i.e. both parents have to carry the thalassaemia gene in order to have affected children. Carriers do not show any symptoms of thalassaemia, and a blood test is necessary to identify carrier status. There are many mass public screening programmes throughout the world which aim to control and prevent this condition. In 2004, Saudi Arabia started a compulsory premarital screening programme to identify thalassaemia carriers, along with carriers of another haemoglobinopathy (sickle cell anaemia). To date no attempt has been made to investigate the Saudi people's thoughts and feelings towards the screening. This qualitative study used a semi-structured interview guide and was designed to explore the attitude of Saudis who have undergone mandatory screening for thalassaemia. Sixteen individuals participated in the study. The primary findings were that: participants accepted and supported the premarital screening programme; their motivations to undergo testing included: having healthy children, reducing the incidence of hereditary diseases in the country, and removing any risk in entering an arranged (usually consanguineous) marriage; the majority of participants lacked knowledge about screening; and many misconceptions and negative feelings towards screening emerged. The main conclusion is that there is a pressing need to increase public awareness about screening advantages and, more importantly, to educate individuals undergoing carrier testing.

## EP07. Disclosure of test results

### (professionals/patients/families/third parties)

#### EP07.1

##### Communicating genetics research results to families: problems arising when the patient participant is deceased

**L. Ormondroyd<sup>1</sup>, C. Moynihan<sup>1</sup>, A. Ardern-Jones<sup>2</sup>, R. Eeles<sup>3</sup>, C. Foster<sup>4</sup>, S. Davolls<sup>1</sup>, M. Watson<sup>1</sup>;**

<sup>1</sup>Psychology Research Group, Institute of Cancer Research, Sutton, United Kingdom, <sup>2</sup>Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, London/Sutton, United Kingdom, <sup>3</sup>Translational Cancer Genetics Team and Cancer Genetics Unit, Royal Marsden NHS Foundation Trust, London/Sutton, United Kingdom, <sup>4</sup>Macmillan Research Unit, School of Nursing and Midwifery, University of Southampton, Southampton, United Kingdom.

**Purpose:** This study explores communication within families of clinically significant genetics research results, after the death of the patient participant. BRCA2 mutations were found in several men after their death from prostate cancer. Spouses were given the results in a genetic counselling session and asked to inform relatives.

**Method:** Cross sectional, qualitative exploratory study. Interviews with 13 relatives, including informers and recipients of the information, were analysed using interpretative phenomenological analysis.

**Results:** Dissemination was hampered when communication channels between relatives were limited, because of family rifts or socially distant or problematic relationships. When informing other branches of

the family, relatives approached individuals in the generation of the deceased man, regardless of their risk status, who were then responsible for informing younger relatives. Most people informed by a relative did not seek genetic counselling. The informing relative may not have sufficient authority for the information either to be taken seriously, or to challenge individual constructions about the aetiology of cancer. This impeded information transmission to further at risk relatives. Most participants knew of relatives who had not been told about their cancer risk.

**Research Implications:** When recruiting participants to genetics research studies, consideration should be given to requesting contact details of specific relatives, in the event of clinically significant results becoming available *post mortem*.

**Clinical Implications:** The limited efficiency of information transfer among relatives is discussed in the context of a potential role for genetics services in contacting at risk relatives directly.

#### EP07.2

#### Exploring family communication after receipt of BRCA1/2 results: Early data from family cases

**C. Jacobs<sup>1</sup>, M. Wallace<sup>2</sup>, J. Smith<sup>3</sup>, S. Michie<sup>2</sup>:**

<sup>1</sup>Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, <sup>2</sup>University College London, Department of Psychology, London, United Kingdom, <sup>3</sup>Birkbeck College, Department of Psychology, London, United Kingdom.

Research investigating family communication about test results for the BRCA1/2 genes has largely been quantitative or limited to investigation with individuals undergoing testing. Little is known about how test information is communicated to other family members or received by them. This study examines how results of BRCA1/2 gene mutations are received by affected index patients and communicated through the family. The study examines the flow of information from clinician to patient and on to relatives; factors influencing communication; the impact of communication on cognition, emotion, behaviour and family relationships; and decision-making regarding risk management.

Results consultations with participants are audio-recorded. One month later, interviews with participants explore their understanding of, and response to, the test result, and the experience of communicating this to first-degree relatives. Similar interviews are then conducted with relatives recruited by the index patients, with whom results have been shared. All interviews are recorded, transcribed and analysed.

Early data from family cases will be presented to illustrate the factors influencing family communication, and the impact of communication on risk perception, emotional state, behaviour and family relationships.

The value of these findings both clinically and scientifically will be discussed.

#### EP07.3

#### Active Non-Disclosure in the Familial Cancer Setting: A Case Report

**M. K. Kentwell, M. Harris, G. Lindemann:**

Familial Cancer Centre, Royal Melbourne Hospital, Parkville, Australia.

A 77 year old woman unaffected by cancer was shown to carry the family specific BRCA2 mutation identified in her sister. At the results disclosure appointment, the client stated she would not inform her four daughters, aged in their 30s and 40s of her positive result.

This presentation will analyse the client and genetic counsellor's perspective, and the interaction between the two. Firstly, the reasons why the client elected not to share her results with her daughters will be explored. These include perception about effectiveness of risk management, family relationships and communication, perceived receptivity of the daughters regarding genetic information, judgement of how the daughters would cope with the knowledge of their mother's BRCA2 mutation carrier status, the perceived harm which may arise from disclosure and personal belief in the daughters' right not to know. Secondly, from the Genetic Counsellor's perspective, the counselling strategies utilised during and following the disclosure appointment will be outlined. In addition, the discussion will reflect on the challenges encountered in counselling the client, and the impact of a consultation involving non-disclosure on the Genetic Counsellor. The presentation will also describe the self-reflection which occurred in attempting to define the Genetic Counsellor's role in this case, and the ethical questions the Genetic Counsellor asked herself around the issue of non-disclosure.

#### EP07.4

#### The psychological impact of genetic testing of symptomatic patients - A literature review

**F. Vansenne, P. M. M. Bossuyt, C. A. J. M. de Borgie:**

Academic Medical Centre, Amsterdam, The Netherlands.

At present more and more genetic risk factors are identified to explain the development of common disease. As a consequence an increasing number of genetic tests are becoming available to clinicians. To guide the appropriate use and clinical utility of genetic tests it is important to consider the psychological impact of testing. In this study we performed a literature review to evaluate the psychological impact of genetic testing and its measurement in symptomatic patients.

We completed a systematic literature search from 1966 to April 2007. Relevant studies were identified from searches of Medline, Embase and Psychinfo databases. Additionally we searched for recent overview articles and relevant reports and in the Health Technology Assessment and DARE database. Prospective studies, evaluating genetic testing in symptomatic patients ( $n \geq 20$ ) and using at least one standardized psychological outcome were included and evaluated.

In total 1233 studies were identified. After abstraction of information 17 studies were eligible and independently assessed. In half of the studies all participants were female, diagnosed with breast and/or ovarian cancer. Socio-demographics were comparable between the studies. Methodology used to measure the impact of genetic testing, protocols, and psychological outcome definitions differed substantially among the reviewed studies.

Little research has been conducted to support the feasibility on when and how to evaluate the psychosocial impact of genetic testing in symptomatic patients. Because of discordant results and short-term follow-up it is uncertain whether patients experience long-term negative psychosocial consequences of genetic testing. This important clinical evaluation question should be addressed in future research.

#### EP08. Predictive testing: process and impact

##### EP08.1

#### Decision making for young adults in predictive genetic testing

**K. M. Mattingley<sup>1</sup>, G. Betts<sup>2</sup>, M. Freckmann<sup>1</sup>, R. Sachdev<sup>1</sup>:**

<sup>1</sup>Clinical Genetics, St George Hospital, Sydney, Australia, <sup>2</sup>Sydney Children's Hospital, Sydney, Australia.

In recent years a number of articles have discussed the predictive testing process in minors and young adults. A young individual's ability to make an informed and responsible decision regarding predictive testing and the potential harm this may cause has been a contentious issue. A more recent view (1) argues that assessment of the individual's maturity of judgement in the decision making process, should be a consideration in predictive genetic testing protocols for minors. Should this not also be applied to young adults? Not all adults by the nature of their age are 'mature' in their decision making ability. Cognitive but also psychosocial factors influence decision making whether in adolescence or adulthood. Legally 'mature' by age may not mean cognitive or psychosocial maturity and can result in immature decision making. Should we then deny young adults who don't have a mature outlook access to testing? The case of three sisters, 18 to 22 years of age, at 50% risk for Huntington disease and their journey through the predictive testing process is presented and discussed.

1. Richards, FH. (2006) Maturity of judgement in decision making for predictive testing for nontreatable adult-onset neurogenetic conditions: a case against predictive testing of minors. *Clinical Genetics* 70: 396-401.

##### EP08.2

#### Accurately assessing uptake of predictive testing for Huntington disease.

**R. J. Tassicker:**

Genetic Health Services Victoria, Parkville, Australia.

The current literature fails to provide an accurate calculation for the uptake of predictive testing for Huntington disease. Uptake figures are reported from several centres based on the total number of people who have undertaken predictive testing as a percentage of those estimated to be at 50% risk in their catchment area. This method produced

a figure of 35% from this state's service, much higher than observation of the local pedigrees indicated. Reworking uptake as an annual figure partly corrects for the length of time a program has been operating. Other identified errors include the use of the cumulative total of those who have tested, with a static denominator of those at 50% risk; and the failure to exclude from the at-risk group those who are too young (ineligible) to test.

Most studies have used population prevalence of Huntington disease to calculate the total of those at 50% risk. We report new data for this state, Victoria, estimating the prevalence to be 8 per 100,000 in 1999. Additional information collated from the Huntington Disease Register of Victoria indicated that for every diagnosed person, there were 4.2 individuals at 50% risk (1:4.2), a lower ratio than the 1:5 hypothesized in the literature. We are currently developing an uptake formula which more accurately incorporates the variables. Our calculation gives an uptake of approximately 13% for this region. An accurate uptake is necessary to assess and interpret testing behavior.

#### EP08.3

#### **Psychosocial genetics: psychological long term family impact (5 years) of the predictive test for Machado-Joseph disease on Azores**

C. D. Gonzalez<sup>1</sup>, E. M. Gomes<sup>2</sup>, M. Lima<sup>3</sup>, C. Bettencourt<sup>3</sup>, T. Kay<sup>4</sup>, J. Santos<sup>5</sup>, J. Vasconcelos<sup>5</sup>;

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**Objective:** Evaluate the long term(5 years) psychological impact in the pre-symptomatic genetic testing(PT) for the Machado-Joseph disease (MJD) in Azores, the motivations for the test and the disease representations.

**Methods:** Scales to measure the individual well-being;family satisfaction, semi-structured interviews of psychosocial assessment and family dynamics.

**Results:** Of the 53 individuals revalued, 92.45% say that would take again the PT, presenting as the main motivation planning their future based on the test results.

In terms of geographic distribution of individuals, its to highlight that in Flores island, all individuals reassessed said that they would do again the PT,maintaining the motivation initially submitted.

Regarding family dynamics, 58.49% of those claim not notice any change, however this information is opposed by the results of the interviews conducted, in which it is noted that 24.53% of these individuals describes changes in dynamics either by approximation or by distancing family.

**Conclusions:** The study shows that most of the individuals who made the PT for MJD would repeat it, considering that knowing the test result contributed to "plan their future."

In terms of family dynamics, it is apparent that changes were focusing on these two opposing poles: the remote and closer family.

Levels of moderate and severe stress in subjects reflect the influence of the results of PT in the psychological well-being of individuals.

It's clear that the results of PT, influence the psychosocial situation of the individuals and their family dynamics, justifying the need for a psychosocial monitoring of individuals and families during the program.

#### EP08.4

#### **Psychosocial evaluation for the predictive test of the Machado-Joseph disease**

C. D. Gonzalez<sup>1</sup>, E. M. Gomes<sup>2</sup>, M. Lima<sup>3</sup>, C. Bettencourt<sup>3</sup>, T. Kay<sup>4</sup>, J. Santos<sup>5</sup>, J. Vasconcelos<sup>5</sup>;

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**Objective:** To publicise the methodology used in the psychosocial evaluation process prior to the test for Machado-Joseph disease of the Azores.

**Methods:** Semi- structured interview, family genogram and psychomet-

ric scales measuring the individual and systemic resilience of those who wish to carry out the Predictive Test (PT).

**Content:** According to one of the objectives of GAIN (Azorean Group of Neurogenetics Research), the continued improvement of the protocol of the Genetic Counseling for the PT, and after the completion of a detailed study of reevaluations of PT's until 2006, which led us to consider the importance of the first interview in psychosocial adjustment to the test result, we started a new methodology in psychosocial assessment of the candidates for the PT.

This approach seeks to identify the different types of these individuals, taking into account the influence of risk factors increased or decreased as the interaction with the individual protective factors and its relational system, making prognosis of a better or worse adapt to the results of PT. In this sense, we've followed a set of criteria which take into account the evaluation of the self, the kind of argument used by individuals and the systemic factors within and outside the family.

This assessment allows the scheduling of sessions of psychosocial counseling, aimed enhance the protective factors and minimize the risk factors prior to the predictive test, for a better psychological adjustment after the predictive genetic information.

#### EP08.5

#### **Psychology of predictive medicine: Acceptance of testing for genetic predisposition to breast cancer**

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Medical Faculty, Sofia, Bulgaria.

This presentation is the closing part of longitudinal study over attitude of Bulgarian people towards the possibilities and achievements of genetic science. The first part that included women, health-care workers(non-genetics) was "Acceptance of testing for genetic predisposition to breast cancer: The attitude of medical professionals."

The second part covered the acknowledgments of medical or biological students and was titled "Acceptance of testing for genetic predisposition of academic youth to PS DNA t for autosomal dominant inherited late onset diseases".

At this third level we expanded our study in the opinion of non-medical healthy people.

In conclusion this study compares the three groups- MD specialists, students and non-medical healthy people mainly towards:

1. Information sources.
2. Attitude of the possibilities of "predictive medicine".
3. Personal choice in genetic case situation.

#### EP08.6

#### **Lessons of uncertainty from a rare inherited neurodegenerative disease: may it be a model?**

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The acronym IBMPFD designates inclusion body myopathy with Paget's disease of the bone and frontotemporal dementia, a rare autosomal dominant disorder caused by missense mutations in the *VCP* gene. The disease usually presents in adult life; no effective treatment is available.

When we encountered the questions and the needs of an at-risk family member, we dealt with a disease characterised by: i) variable involvement of multiple systems; ii) age-dependent, yet unpredictable, penetrance of each of the cardinal signs; iii) possible occurrence of progressive cognitive decline. The co-presence of these features makes IBMPFD a compelling model for genetic counselling, as it resembles a complex disorder despite the Mendelian mode of inheritance.

The recommendations for predictive testing published for familial dementias state that the protocol developed for Huntington's disease (HD) should be followed. However, the deterministic scenario prompted by the HD mutation may appear simplistic when we face a number of additional uncertainties, including: i) the inability to predict the clinical presentation, i.e. the involvement of either muscles, bones, brain, or all; ii) the low predictive value of a negative test result, due to the high prevalence of cognitive decline among aged individuals. Nevertheless, it can be inferred from literature that predictive testing was performed in families included in research protocols, although the genetic counselling procedures were not mentioned. We will address some of these issues, by comparing the theme of multiple uncertain-

ties raised by IBMPFD with the evidence from the genetic counselling protocols available for other neurodegenerative and neuromuscular disorders.

#### EP08.7

#### The role of the disease in the psychological impact of presymptomatic testing for SCA2 and FAP ATTRV30M: knowledge of the disease in the family, degree of kinship and gender of the transmitting parent

*M. Panque Herrera<sup>1,2</sup>, C. Lemos<sup>1</sup>, L. Velázquez Pérez<sup>2</sup>, J. Sequeiros<sup>1</sup>, M. Fleming<sup>1</sup>;*

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To study factors of psychological impact of presymptomatic testing (PST) of spinocerebellar atrophy type 2 (SCA2) and familial amyloid polyneuropathy (FAP ATTRV30M), we analyzed (i) the effect of previous experience with the disease in the family, kinship with closest affected relative and gender of transmitting parent, when adapting to test results; and (ii) differences in the course of psychological wellbeing in 63 subjects, 28 offspring at risk for FAP, Portugal, and 35 at risk for SCA2, Cuba (who up-took testing May 2004 to April 2006).

Persons with less previous knowledge of the disease in the family referred more anxiety; lower levels of anxiety and depression were seen when the disease was present in first-degree relatives; having an affected mother was associated with lower levels of depression, both immediately and one year after results. Offspring at-risk for FAP had less anxiety than those at-risk for SCA2, during the whole follow-up (1 year), though differences were not significant.

A longer period of contact with the disease, closer kinship and an affected mother all lessen the impact of PST, as expressed in levels of anxiety and depression.

#### EP08.8

#### Is emotional impact of genetic testing related to the subjective risk to be a mutation carrier? The example of neuroendocrine tumors

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Paraganglioma and pheochromocytoma are generally benign neuroendocrine tumors, inherited in about 25% of cases (autosomal dominant model), causing by germline mutations in SDHD, SDHB, SDHC, VHL, RET or NF1 genes. The hereditary form of the disease is characterized by an early onset with a higher risk of recurrence and/or malignancy. Genetic testing of patients and their families opens to earlier diagnosis and treatment of asymptomatic tumors in mutation carriers.

Objective: To evaluate emotional impact of genetic testing in consecutive patients met during oncogenetic multidisciplinary consultation dedicated to pheochromocytomas and paragangliomas.

Methods: Baseline state and trait-anxiety (STA1), depression (BDI-13) and subjective risk to be career of the mutation were assessed before the blood sample. A second assessment of state-anxiety, depression and traumatic impact of the announcement (IES-R) was performed after the definitive test result.

Results: 29 subjects were tested and 22 received the definitive result (12 positive, 10 negative). Baseline depression was correlated with the number of children ( $\rho=0.40$ ;  $p=0.03$ ). There was no change in state anxiety and depression after the test result. Psychological scores were not associated with subject's status (index case or relative). State-anxiety, depression and impact of event scores did not differ according to the test result. A higher impact of the result was found when subjects expected to be carriers whereas they actually were not ( $p<0.05$ ).

Conclusion: Our data encourage to evaluate the subjective risk to be a mutation carrier before the test and to be particularly careful when the result is opposite to the subjective risk.

#### EP09. Predisposition to common diseases: genetic testing and preventive behaviour

#### EP09.1

#### Women's attitudes towards genetic susceptibility screening for breast cancer to target disease prevention

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<sup>1</sup>VU University Medical Center, Amsterdam, The Netherlands, <sup>2</sup>EMGO Institute, Amsterdam, The Netherlands, <sup>3</sup>Dept. of Clinical Genetics, Amsterdam, The Netherlands.

Currently, breast cancer screening is offered to all women based on age, usually beginning at age 50. It has been suggested that susceptibility testing for breast cancer genes may be used for risk profiling to target interventions (i.e. mammography screening) only to those at higher risk. The aim of the study was to explore women's attitudes towards population genetic susceptibility screening to target breast cancer prevention.

Four focus groups were conducted with 26 women aged 40-73 years. Women were selected irrespective of personal or family history of breast cancer.

The results show that in general women are positive towards genetic risk profiling for breast cancer, provided that in the lower risk group, though maybe less frequent, women are still offered mammography screening. Women reported that they would expect that some women would find it difficult to cope with knowing to be at higher risk. Others believed that in this way women would be able to change their lifestyle or start screening at earlier age. Bottlenecks mentioned by the women were potential problems with insurance, expected high costs of testing, putting too much emphasis on genetics. Women recognized that this risk profiling was just for one disease, but were ambiguous whether or not it should be extended to other diseases.

In conclusion, these results suggest that women currently offered breast cancer screening based on age, accept a possible future susceptibility screening for breast cancer, but also identified issues that need to be discussed and studied further.

#### EP09.2

#### Health behaviour after genetic counseling: A population-based study

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Background: Alcohol consumption and obesity are known risk factors for breast cancer. Together with other health behaviours, such as physical exercise and smoking, they may also influence the risk of hereditary breast cancer. To date, however, very few studies have addressed the impact of genetic counseling on any of these health behaviours.

Purpose: To assess the impact of genetic counseling on health behaviours, in terms of smoking, physical exercise, alcohol intake and diet habits.

Method: A population-based prospective cohort study including women (N=319) who received their first genetic counselling for hereditary breast and ovarian cancer risk, and two reference groups. Reference Group I consists of women (N = 417) who received mammography. Reference Group II consists of women (N = 1,271) randomly selected from the Danish population.

Data were collected by standardized, questionnaires mailed prior to counseling or mammography and 12 months post-counseling.

Results: Findings on smoking, physical exercise, alcohol intake and diet habits will be presented and discussed.

## EP10. Genetic counselling: communicating genetic information

### EP10.01

#### Pitfalls in communicating 5-Probe FISH results- Lessons learned in genetic counselling

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In Victoria Australia, all pregnant women are offered serum screening to identify those fetuses which are considered to be at an increased risk for Trisomy 21, Trisomy 18 and Neural Tube defects. Women returning an increased risk may be counselled by their general practitioner, obstetrician, midwife or genetic counsellor, and are offered diagnostic testing. Many choose to have a FISH analysis as part of the diagnostic testing.

I present a number of women who experienced a normal FISH result from diagnostic testing, many believing that this was the complete result. They were later found to have a (different) abnormal karyotype-an event that caused distress and confusion.

I discuss their varied reactions and emotions in response to this "changed" result, and how they addressed the unexpected and difficult decision-making that followed.

Examination of these cases highlights the need to exercise great care when giving and discussing prenatal FISH results in the setting of increased risk for aneuploidy via serum screening. Clearer information given when the test is offered may alleviate these situations, but does little to reduce anxiety. The cases also demonstrate a need to provide ongoing education for health professionals who offer these tests to their clients, often also believing that the normal FISH result is an accurate description of the final result.

### EP10.02

#### Family Perspectives on Inherited Arrhythmia Services

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Assessing the interests and experiences of individuals affected by genetic conditions is an important component of service development. In the genetic arrhythmia literature, there is a paucity of information about what both affected and at-risk family members feel are the important elements of their care. We present the results of an anonymised survey administered to patients and their relatives who have been assessed for genetic arrhythmia through the CHEO Inherited Arrhythmia Clinic. A response rate of 52% was achieved with online and postal surveys. The respondents were 55 individuals (45% male) from 25 families, aged 14 to 70+ years who attended the clinic because of diagnosed or suspected long QT syndrome, arrhythmicogenic right ventricular cardiomyopathy, or catecholaminergic polymorphic ventricular tachycardia. Results: Respondents perceive education and protective follow-up care to be the most important roles of an inherited arrhythmia service. Healthcare decision-making and support also emerged as important roles. At the first appointment, primary information needs concern the heart condition itself, long-term outcomes, and restriction of activities. Support group information is a low priority initially, but emerges later as an important requirement. With respect to timing of genetic counselling, respondents indicated no strong preference for counselling in advance, same-day or after heart investigations. However, 70% of respondents expressed that the location of counselling matters; the majority expect face-to-face counselling in a private location. These findings provide insight into families' preferences for inherited arrhythmia services, and how such services might be most optimally configured to meet their needs within inevitable resource constraints.

### EP10.03

#### What physicians tell to individuals at risk of breast and ovarian cancer syndrome (HBOS) referred for genetic counselling. IMASS Spanish cohort

**E. Darder, B. Graña, A. Velasco, D. Fortuny, T. Ramón y Cajal, A. Torres, J. Sanz, C. López, N. Gadea, I. Mensa, C. Saura, J. Brunet, J. Balmáñia; IMASS, Barcelona, Spain.**

**Background:** Individuals at risk of HBOS are referred to genetic counselling units for genetic testing. It is unknown how much information these individuals get by their reference physicians before counselling. **Materials and Methods:** Spanish individuals at risk of HBOCS referred to genetic counselling were asked to participate in a multicenter study by fulfilling a self-completed questionnaire before *BRCA1/2* testing. Variables were analysed using descriptive statistics and Fisher Exact test with SPSS.

**Results:** Overall, 243 individuals were enrolled. Median age was 44 years (range 19-88), 88% were women and 67% had a previous diagnosis of cancer. Most participants (65%) were referred by a physician, 30% by a relative and 5% were self-referred. The reference physician did not inform 48% of individuals about their risk of developing cancer, of those informed 70% were told to have a higher cancer risk than the average population. Physician informed 46% of total individuals about the possibility of genetic testing recommending it to 37%. Seventy-five individuals (31%) did not receive any information about genetic counselling/testing before their risk assessment visit, while the main information source for 74 (30%) was the media. A similar proportion of cancer patients and healthy individuals at risk of HBOS received previous information by the reference physician (53% vs. 49%, p=0.58).

**Conclusions:** A low percentage of individuals at risk of HBOS are informed by their reference physician about their cancer risk and genetic predisposition before genetic counselling. Further efforts have to be done to improve medical awareness about hereditary cancer syndromes.

### EP10.04

#### Cancer Knowledge, Risk Perception, and Cancer Worry after Genetic Counselling for Hereditary Breast Cancer in Spain.

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Identification of a genetic basis underlying breast cancer has led to an increase in demand for genetic counselling about individual risks of the disease. Genetic Counselling Units are becoming an integral part of cancer services. It is, therefore, important to assess how attendance at these clinics impacts on cancer knowledge, cancer-related concerns, and risk perceptions. Previously, we have reported a validation of a Spanish Translation of the Cancer Worry Scale, Escala de Preocupación por Cáncer, which can be used to asses cancer worry in the setting of genetic counselling. A total of 212 Spanish patients were consecutively recruited, and 152 completed all questionnaires before and one and 6 months after genetic counselling. Cancer knowledge, including breast cancer prevention measures, significantly increases after genetic counselling. Patients' risk perception did not correlate with the actual breast cancer risk calculated by the counsellors. Patients' perceived risk did not modify after genetic counselling. Patients were significantly less worried after counselling. Higher levels of worry were predicted by high degree of perceived risk of developing cancer, higher levels of anxiety and low level of education. In conclusion, counsellors met the patients' psychological needs to a satisfactory degree during counselling. However, patients did not fully understand their risk of developing cancer.

### EP10.05

#### Breast Cancer Genetic Counselling in Cyprus: First epidemiological data

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*ics, Nicosia, Cyprus, <sup>4</sup>Bank of Cyprus Oncology Center, Nicosia, Cyprus.*  
Cancer Genetic Counselling in Cyprus was established recently. Breast cancer patients, account for the majority of referrals at present. They are referred to the Cancer Genetics Clinic based on age of diagnosis, type of cancer and family history. Referred patients are offered two counselling appointments to be appropriately informed of the risks, benefits, and limitations prior to decision making for testing. Results are communicated through a further appointment which is followed by other counselling appointments according to the needs of the patients and their family.

In 2007, 103 breast cancer patients and healthy relatives at risk were seen at the CGCC. We analyzed the variability within this patient group in terms of demographic information.

The 101 female and 2 male patients were mainly referred by their oncologists. In terms of age, 4 were < 30, 42 were 30-39, 29 were 40-49 and 28 were > 50 yrs. 97 were Greek Cypriot, 1 was Turkish Cypriot and 5 were of other ethnic backgrounds. Genetic counselling for cancer predisposition genes was in general well appreciated. Uptake of genetic testing by patients and their family members were diverse. These and other data are being analyzed currently and will be reported in this poster.

#### EP10.06

#### The increased incidence of breast cancer makes obligatory the genetic counseling

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In our days there are more and more cases of different cancer types. We can mention many causes responsible for the appearance of cancer: environmental, chemical, physical and biological factors. Of course, in many cases the inheritance has an important role in appearance of different types of cancer. Our study is about the dynamics of cancer in our county during the 1990-1999 period and the necessity of genetic counseling of the persons who have cancer affected relatives. We analysed the data from Medical Direction of Bihor county in mentioned period. We made a statistical study about the incidence of breast cancer in our county. Also we show the stages for genetic counseling in two types of groups (one already breast cancer affected and one with relatives affected).

We studied the incidence of breast cancer in feminine population. Also, we studied the area of proceeding and type of cancer. The stages in genetic counseling in breast cancer must include: family and medical history, hereditary vs. sporadic cancer, genes associated with cancer, risk assessment, testing options, test results, screening options, possible prevention options and psychosocial assessment.

The breast cancer has an increased incidence in Bihor county. The incidence depends in some types of cancer on patient sort (masculine or feminine), age and proceeding area. Because of these, genetic counseling is very important in prevention of this type of cancer.

#### EP10.07

#### Communicating genetic information: learning from patient experiences and preferences

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Where do patients get information about the genetic basis of their condition? What healthcare professional groups outside specialist genetics services provide genetic information and what kinds of information are given? How would patients prefer to receive such information? A qualitative study was conducted in the UK in 2006 to explore these issues, through telephone interviews with 27 people with or at risk of genetic conditions and parents of children affected by a genetic condition.

The results highlight the range of healthcare professionals who may be approached by patients seeking information and indicate a perceived need for greater awareness of genetic aspects of conditions amongst healthcare professionals. Patients acknowledged that healthcare professionals cannot be familiar with all genetic conditions in detail, but felt that identifying and referring patients appropriately, and acknowledging professional limitations of expertise, were important.

Patients gave us their views on the knowledge, skills and attitudes

which they believed would enhance care by non-genetics professionals. They valued certain attitudes: providing genetic information without judgement; being mindful of the use of terminology; and being aware of the emotional impact of genetic information on individuals and the wider family. Tailoring the information provided to individual preferences and informing people where they can access further information were also considered important.

These results have implications for the education and practice of all health professionals involved in providing genetic information. They are being used in the UK to raise health professionals' awareness of genetics and to inform the integration of genetics into professional training.

#### EP10.08

#### Evaluating Service Development: a community based genetic service for consanguineous families of South Asian origin

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A UK Department of Health service development project developed community based, hospital linked genetic services for Asian families affected by autosomal recessive conditions. The 2 year pilot project (2005-2007) took place in Blackburn a town in the North of England where 20.4% of the population are of Asian Origin, 50-60% marriages are consanguineous and there is a high incidence of autosomal recessive disorders. The aim was to provide a culturally sensitive service by an Asian speaking genetic counsellor. An evaluation determined how genetic information was understood, received and transmitted within these families and factors that affected decisions about genetic testing and sharing of information.

A total of 42 families affected by a range of autosomal recessive disorders were included. On average 5 extended family members were seen from each index family referred. Uptake of the service was 95% with positive feedback from families.

Evaluation Key points:

- 97% of families found the service useful and informative.
- The number of requests for prenatal diagnosis and carrier testing demonstrates utility.
- Families identified language and a lack of understanding about Genetic services as previous barriers to accessing services.

Key outputs:

- development of protocols/care pathways to access appropriate family members.
  - establishment of a community based hospital linked genetic service.
  - contact with families previously unknown to clinical genetics service.
- The paper will highlight families' views and feedback on the service and make recommendations for future practice.

#### EP10.09

#### Attributions as predictors for level of functioning and depression in cancer patients

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In this study we aimed at analyzing whether a negative attributional style is an efficient predictor, for both the male and female patient's future evolution, depending on their stage of illness and type of diagnosis. Our main objective was to uncover changes and reactions at the psychological level of the patients during their stay at the oncology ward or during medical interventions. By interviewing the patients (before we administered specific instruments based on the learned helplessness theory, such as the Attributional Style Questionnaire) we gathered information regarding their level of functioning which led to the formation of three independent groups.

Patients diagnosed with cancer (overall 120 subjects) who did not show any symptoms of mental disturbance, 67 females and 53 males who were diagnosed with cancer, classified as having stage one and two, and 36 stage three and four. A total of 79 patients suffering from depression were included in the study, 34 males and 45 females.

Regression analysis showed that the negative attributional style is an efficient predictor for depression in the case of patients suffering from cancer of the digestive tube. The negative attributional style is a very

efficient predictor for the level of functioning and perceived pain in the case of patients suffering from pulmonary cancer and digestive tube. Overall, the results show that the negative attributional mechanisms coupled with other socio-cognitive variables can increase the level of depression for patients diagnosed with cancer, on the background of lack of control and / or deficient functioning.

#### EP10.10

#### An international online survey of genetic health professionals' practice involving family communication

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Communication of genetic information in families is becoming increasingly important due to the low numbers of at-risk family members that contact genetic services for counselling. Genetic counsellors and clinical geneticists (genetic health professionals) are responsible for educating and discussing the familial implications of a genetic diagnosis with probands and consultands. However, genetic health professionals' practice in family communication is largely unexplored.

This is the first international survey to be developed and validated which aims to explore genetic health professionals' current practice involving family communication. The survey was administered online and participants were recruited through the membership email lists of organisations with clinical geneticists and genetic counsellors as professional members.

The survey was completed by 628 genetic health professionals. The results demonstrate that the majority of genetic health professionals always identify which relatives are at-risk (95.6%) and encourage communication about the genetic condition to these family members (95.4%). There were generally no differences between clinical geneticists and genetic counsellors practice ( $p>0.05$ ) when counselling probands about at-risk relatives, except when discussing which relatives are at-risk of developing Huntington disease ( $p=0.02$ ). Genetic health professionals' practice did not vary greatly when compared across four scenarios which each involved a genetic diagnosis of haemochromatosis, familial adenomatous polyposis, Huntington disease or a balanced reciprocal chromosomal translocation.

The results of this survey provide an insight into genetic health professionals' practice internationally and provide information for the development of evidence-based practice for genetic and non-genetic health professionals in the area of family communication.

#### EP10.11

#### Genetic Counseling in the Muslim World : The Challenges

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Genetic counseling is the process in which an individual or a family obtains information and advice about a genetic condition that may affect the individual, his progeny, his relatives, or the family as a whole. Based on this knowledge he can take the pertinent decision regarding marriage, reproduction, abortion and health management.

Genetic counseling includes five themes, medical management, risk determination, risk options, reproductive decision making, and support services. It involves a partnership of physicians, genetic counselors, and genetics support groups. The majority of clinical geneticists subscribe to the principle of non-directive ness: information about risks, natural history, treatment, and outcome are presented in a balanced and neutral manner, but decisions about reproduction are left to the family.

Public health authorities are increasingly concerned by the high rate of births with genetic disorders especially in developing countries where Muslims are a majority. Therefore it is imperative to scrutinize the available methods of prevention and management of genetic disorders. In the Muslim World and in the Kingdom of Saudi Arabia (KSA), genetic counselling involves many challenges, as it has to be carried within the context of religion and culture, according to Islamic ethical and cultural background of the individual, with community-based genetic counseling in one's own language, in the presence of paucity of expertise, resources and technology.

#### EP10.12

#### „Do you know why the doctor sent you?“ - Characteristics of the genetic counselling process in a multicultural antenatal context in Johannesburg South Africa

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Few genetic counselling (GC) research studies have examined the GC process itself, particularly within multicultural settings. State-funded antenatal GC clinics in South Africa service culturally diverse populations and this research study aims to investigate the nature of these encounters. Using qualitative methodology, the required data is obtained from GC sessions and post-session interviews with genetic counsellors and the women using the service. GC sessions are video recorded, transcribed and analysed using thematic content analysis. The results of three sessions from two counsellors will be presented. Initial findings suggest that there is an almost standard structured GC format used by counsellors which includes: establishing patient's expectations, explaining GC role, obtaining information, providing information, facilitating decision-making and making referrals. Time spent on each aspect varies according to the women's understanding of language and content (as assessed by the counsellor), depth of emotional engagement and counsellor's skills of communication and relationship building. Analysis of the nature of the sessions shows some themes emerging and these include: encouraging discussions, attempts to connect, clarifying techniques, simple language and patient beliefs. This initial analysis showed heightened counsellor awareness of the need to use comprehensible language to convey concepts and counsellor willingness, but difficulty in eliciting and responding to emotional content in the face of language and cultural differences.

#### EP10.13

#### Evaluation of genetic counselling in a hospital-based clinical service

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In order to ascertain the effectiveness of genetic counselling it is necessary first to evaluate how it is carried out and identify aspects that need to be improved. There are few published studies on this topic. Our aim was to evaluate the genetic counselling provided by the Genetics Service of the Vall d'Hebron Hospital in Barcelona. Seventy-four out of 108 cases/families (68%) seen in clinic from February to December 2007 were eligible and agreed to participate in this study. Each participant answered a questionnaire before and after the consultation.

The whole survey was developed taking into account previous work and publications on the evaluation of genetic counselling. We were particularly interested to study the patient's prior expectations and whether they had been met during the consultation, patient's satisfaction (instrumental, affective and procedural aspects), and the level of comprehension of genetic and medical information given during the consultation. Other epidemiological data, such as gender or age, were also collected. Subsequently, a statistical analysis was performed. Participants were satisfied with the genetic counselling received (mean score of 3.42 / 4, equivalent to 85.5% maximum satisfaction), and their expectations were met in 79%. They answered correctly 84% of the questions about medical and genetic information given to them by the geneticist during the consultation. In summary, the evaluation of the genetic counselling service was globally quite positive and allowed to identify some aspects that could be improved.

#### EP10.14

#### Clinician-patient interaction during genetic consultation and counselling - Case study in five genetic clinics in Colombia

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In Colombia and Latin America, genetics services are relatively new, and little attention has been paid to the critical process of communication during genetic consultation and counselling.

In collaboration with the Bogota Health Service (Ministry of Health and Social Protection of Colombia), the Medical Genetics group of the Colombian Association of Human Genetics, and the University of Warwick (UK), we observed 25 genetic consultations in Colombian genetic clinics and undertook semi-structured interviews with the participants before and after the consultation. Thematic analysis of the interview transcripts demonstrated widespread mismatches between practitioner perception and patient comprehension. Effective communication was inhibited by patient, family, practitioner and environmental factors. Principal among these were excessive administrative procedures, interruptions during the consultation, patients' lack of attention to medical terminology, excessive information given in one session, beliefs and education level of the patient and/or relatives, patient distress caused by bad news, unfulfilled expectations and no availability of treatment. We also interviewed 20 medical consultants working in genetics services. There was general agreement that genetic counselling in Colombia was problematic, and that more training in communication skills was required at Medical schools. Many physicians did not believe that other health professionals should work as genetic counsellors. There was a widespread recognition of limited genetic knowledge in most medical specialities.

These findings will inform the future development of an effective and robust genetic counselling service in Colombia. They will also be used in the development of the academic curriculum related to basic and clinical genetics at Colombian Universities.

#### EP10.15

#### Communication of genetic information in families: Funding for a randomised controlled trial of a genetic counselling intervention

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When a person receives a diagnosis of a genetic condition for themselves or for their child, many of their at-risk relatives remain unaware of this information. This has important health implications, at present usually in families with single gene disorders, but in future for complex disorders and pharmacogenomics, toxicogenomics and nutrigenomics. How should important information that will reduce morbidity and mortality be communicated?

The aim of this study is to investigate whether a genetic counselling intervention will result in increased access to genetic services by family members at risk of serious genetic conditions. We have been funded to conduct a randomised controlled trial to assess the effectiveness of intense genetic counselling follow-up on the numbers of at-risk relatives utilising genetics services. The intervention will be developed using appropriate theoretical approaches such as family communication and family systems theory. Clients presenting to a clinical genetic service for diagnosis or genetic testing will have the number of at-risk relatives recorded. Following randomisation into an intervention or control arm, the number of those at-risk relatives who utilise genetics services will be compared.

This multi-disciplinary study will determine whether a genetic counselling intervention improves information exchange in families while also exploring the issues that arise. The ultimate aim of this study is to provide evidence to enable best practice both nationally and internationally for the accurate and efficient communication of genetic information in families for the promotion of their health and well being.

#### EP10.16

#### Mutations of self-other relations in genetic counseling discourse

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Drawing upon the seminal work of George Herbert Mead which underscores how the 'self' is conceptualised as a socially situated reflexive process made possible through the perception of alterity, we propose that the notions of self and other can be understood at a relational level along three possible configurations - 'self-as-other', 'self-and-other' and 'self-vs-other'. The counselling and therapeutic settings, with their narrative and reflection orientation, give primacy to the 'presentation' and 'performance' of the 'self', as can be argued from a Goffmanian

dramaturgical perspective.

Based on a theme-oriented discourse analysis of over 50 audio-recordings of genetic counselling sessions covering a range of conditions, we suggest that different familial lines are mutated along self-other categorisations, reflecting not only the genetic status of the individual concerned but also the trajectories of past and present familial relations. This means that decisions to test and decisions to disclose test procedures/results have to be other-oriented. As far as an individual's genetic status is concerned, the 'carrier' status of a family member may necessitate a different self-other orientation when compared with someone's 'affected' and 'at-risk' status.

In conclusion, we argue that in genetic counselling, both counsellors and clients have to be other-oriented by 'decentering the self' while balancing self-other relations by warranting, explicitly, situated differences and contingencies. The counsellor by seeking 'other' perspectives becomes other-oriented, which simultaneously makes the client other-oriented. Such a stance conflates, following Mead, the 'self-as-other' and 'self-and-other' positions.

#### EP10.17

#### To develop and translate a set of leaflets for patients and families across Europe, that provide information on issues related to genetics and genetic testing

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Aim: To develop and translate a set of leaflets for patients and families across Europe, that provide information on issues related to genetics and genetic testing (part of the EuroGentest project).

Leaflet development: A set of 11 patient information leaflets were developed on topics related to inheritance patterns and genetic testing. The themes of the leaflets were decided by a panel of professionals and patient group representatives. The content of the leaflets was developed with input from patients as well as by building on existing written patient information. After the first draft the content and layout was tested out with patients to ensure readability and ensure key topics were covered. When the content was finalised it was checked by professionals to ensure accuracy of the text and images.

Leaflet translation (currently underway): First, the content is checked with genetic healthcare professionals in each country through a specifically designed questionnaire. Adaptations are then made to the text to ensure it is 'country specific'. Translators are all bilingual genetic professionals or PhD students who are recruited through universities and genetic departments. Translations are cross checked by a second bilingual genetic professional to ensure their accuracy. Leaflets are then uploaded onto the EuroGentest website [www.eurogentest.org/patients](http://www.eurogentest.org/patients) to be freely downloaded by professionals and patients. Leaflets are currently available in 8 languages, with 12 more to follow. A new set of leaflets which will focus specifically on the psychosocial aspects of genetic testing are currently underway.

#### EP10.18

#### I say, you hear: What do Interpreters say when there are no words.

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Monash Medical Centre is a busy hospital serving a multicultural population in excess of one million people. Many clients are young refugees and couples keen to have children now they have reached Australian "safety".

All pregnant women are offered serum screening for Down syndrome, Trisomy 18 and neural tube defects. Women receiving an increased risk for any of these conditions are referred for genetic counselling to discuss their risk, the condition in question, and their choices.

Interpreters greatly assist our efforts to provide genetic counselling and choices to these migrant couples, but it has become clear that there are times when the clients simply do not understand what the central issues under discussion are. This raises concerns that the decisions made may not have a sound base. Terms such as risk, chance, chromosomes, Down syndrome and spina bifida are a mystery to some.

In order to improve genetic counselling practise, interviews were conducted with hospital interpreters to elicit what is said, especially when

there is not a corresponding word in their given language. Interviews consisted of standard questions as well as an exploration of generally held beliefs about inheritance, and how decisions are made in the family culture.

A review of the questionnaire and the outcomes of these interviews will be discussed.

#### EP10.19

##### The genealogical tree is a very important stage in genetic counseling of the families with mentally retarded children

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In humans, some traits are abnormal and they have diverse etiologies. Certain traits appear isolated in a person and others appear associated in a diseases called syndromes.

To make a pedigree of an individual or a family, it's a necessity to gather data about the family or individual. We investigated 600 children hospitalized on period of 2000-2002 in Neuropsychiatric Infantile Section of Neurology and Psychiatry Clinical Hospital from Oradea.

#### Results and discussions

In 600 children that were examined, 397 presented different levels of mental deficiency. We made family investigations and genealogical tree.

More than 65% of children with mental deficiency have one or more affected relatives in family. The relatives may be affected by congenital abnormalities and/ or mental diseases. The incidences of affected relatives are important in groups with mild and moderate mental deficiency. In the group with severe mental deficiency, the incidence of affected relatives is lower.

This result may be an argument for the hypothesis that genetic factors are very important in the inheritance of mental deficiency. It seems that, elementary, severe mental deficiency appears because of genes and chromosomes disorders, and secondary because of dominant or recessive inheritance. The data about family are systematized in pedigree or genealogical tree of the family. Analysing the pedigree of a family, we can say that some traits are inherited or not. Also, we can anticipate some normal or abnormal traits of individuals of the next generation.

#### EP10.20

##### Psychological and familiar impact of genetic diagnosis in oculopharyngeal muscular dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset, slowly progressive muscular disease. Inheritance is autosomal dominant; penetrance is full, age-dependent. Symptoms are paresis of extraocular eye muscles, dysphagia and limb weakness. Because it is not considered a very disabling disorder and molecular diagnosis is simple - making it easily accessible to almost any laboratory- testing may be requested without much concern about psychological and social aspects. Psychological and familiar impact of this diagnosis has not been tackled. Out of 76 patients with suspected OPMD seen in our unit, 45 (29 families) were positive. In families referred for genetic counselling we carried out a psychological evaluation with a semi-structured in-depth interview analyzing motivations, attitude and anxiety. We found an average of 1.8 secondary cases (symptomatic or asymptomatic) per index case. 82 % of predictive analyses were positive. We received no requests for antenatal diagnosis. Twelve months after genetic testing another psychological exam was done using HAD and a semi-structured in-depth interview to assess personal and familiar impact of the diagnosis. Although molecular confirmation of OPMD did generally not cause serious distortion in psychological aspects or in the familiar core, some aspects of family life were indeed modified. Even if not perceived as a severe disease in their relatives, for many individuals the possibility of a predictive diagnosis caused significant stress. A protocol for

genetic counselling and psychological assessment are fundamental in this disease. Reasons for the low proportion of secondary cases may be multiple and should be further investigated in the future.

#### EP10.21

##### Sense Making in Predictive Genetic Testing of Hereditary Cancer. An Interview Study

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Purpose: Mankind involves an active effort to find a purpose in the events that surround us. This construction of meaning is a process that is reinforced with stressful events that may be inconsistent with our beliefs about when and why things happen. The aim of this study was to evaluate, from a narrative perspective, how biographical factors may contribute to the formation of different meanings of the genetic diagnosis .

Methods: Three pairs of siblings (n = 6) identified as carriers of a genetic defect were evaluated using semi-structured interview designed to identify vital changes associated with the process of genetic counseling. Three independent researchers conducted content analysis and identified significant differences between each pair of interviews by consensus.

Results: In pair of siblings A, the presence of other stressful events minimize the potential harmful of the genetic diagnosis experienced by one of the brothers; in pair B, the relationship with a very affected close relative encourages active coping problem with one of the members; and in C, the concept of brotherhood and sacrifice allow one of the sisters assume their genetic alteration as an opportunity to increase their family privacy.

Conclusions: Despite sharing diagnosis, family history of cancer and genetic counseling process, the narratives of the siblings presented significant differences between them. The use of different frameworks of interpretation according to the biography of each subject provides the genetic diagnosis of sense and different consequences.

#### EP10.22

##### Delivering genetic counselling via telehealth: practitioner's experience of a virtual consultation

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Telehealth, or videoconferencing, is an evolving field in general and cancer genetics. The aim of this study was to qualitatively explore clinicians' perspectives and experiences of delivering telehealth genetic counselling. Semi-structured interviews were conducted with geneticists and genetic counsellors. The interviews explored experience, satisfaction, aims of the service, advantages and disadvantages of the technology, and roles within the consultation. RESULTS: Fifteen practitioners participated. They reported that telegenetics increased staff efficiency and accessibility to outreach clinics. The geneticists presented as the consulting specialist, delivering medical information and screening advice and depended more on the genetic counsellor to assess non-verbal behaviour and subtle emotional cues from the client. Consultations were described as being more formal, and possibly less open to emotional expression than face-to- face consultations. Interactions on-screen were moderated by the physical positioning of the genetic counsellor and client. When the counsellor was positioned "off screen", a medically modelled dyadic interaction occurred and non-verbal cues between the counsellor, the geneticist and the client were obscured. When positioned "on screen", counsellors reported they offered a higher level of psychosocial support before, during and after the telehealth session.

Practitioners were highly satisfied with telegenetics but acknowledged the trade off involved in the geneticist not being physically present. The technology is efficient and offers sufficient resolution to attend to

most patients' psychosocial needs. Findings highlight the benefits of co-facilitation between geneticists and genetic counsellor when each has clearly defined complementary roles.

#### EP10.23

#### Explaining X-linked inheritance: the importance of the personal drawing

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An explanation of the mechanism of inheritance is a key component of many genetic consultations. However, no evidence base exists for this significant area of practice.

We present the results of a process study, involving twenty-one individuals with a family history of an X-linked condition. Their genetic counselling consultations were videotaped and participants were then visited at home by the researcher. Adapting techniques of Interpersonal Process Recall, the section of videotape featuring the explanation of inheritance was played back to participants. Their responses and reflections were elicited and recorded on audiotape. In a separate arm of the study, the counsellors were shown the videotape and were similarly interviewed by a second researcher about their experience. The patients felt that a personalised diagram, drawn by the counsellor during the consultation, conveyed X-linked inheritance in a visual and engaging way, allowing them to conceptualise risk figures. Its step-wise construction facilitated slower assimilation of information, allowed room for questions and assisted with retention of the information. The diagram's individualised nature allowed patients to contextualise their family history such that personally relevant questions could be answered. Importantly, this did *not* appear to require patients to first understand complicated genetic concepts such as the biological basis of genes or chromosomes.

Counsellors too reflected on the construction of a drawing during the consultation; which they felt helped them to both pace and individualise the explanation.

This presentation will elaborate on patient and counsellor reflections on this key aspect of the genetic counselling consultation.

#### EP10.24

#### Explaining X-linked inheritance: the importance of the counsellor-counselee relationship

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Previous research has highlighted that the quality of the relationship between genetic counsellor and counselee can impact on a successful outcome of genetic counselling. Many studies have focused on the counselee's perspective. We report on a process study involving 2 experienced genetic counsellors, as part of a triangulated study looking at how X-linked inheritance is explained in the genetic clinic. Following 10 clinic consultations, the genetic counsellors and the researcher (SP-S) watched the relevant sections of the video together, and counsellor comments were audiotaped and transcribed.

The counsellors recognised a favoured sequence for explaining X-linked inheritance, whilst holding a goal of tailoring the explanation. This was made difficult where rapport was less well established because of lack of verbal/non-verbal cues from the patient or whether it was the first contact. Where the counsellors felt a good rapport had been achieved, they felt better able to take account of age/educational background/need for information in order to personalise the explanation more effectively.

In a separate arm of the study, counselees also expressed the importance of the relationship in giving them confidence- both in themselves to ask questions for clarification- and in the counsellor's ability to help them achieve an understanding.

It is noteworthy that both counsellors and counselees reflected on counselling dynamics during the part of the consultation, which would be thought of us primarily educative. This paper will present further evidence that a division between education and counselling goals in

genetic counselling consultations may be unhelpful.

#### EP11. Strategies to facilitate decision making in genetics

##### EP11.1

##### The impact of a BRCA support-information group on the choice for a preventive mastectomy is limited

**K. M. Landsbergen<sup>1</sup>**

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**Introduction:** Important aims of a BRCA group are assimilation of being BRCA1/2 carrier, and professional guidance in choosing prophylactic mastectomy or breast cancer surveillance.

**Aim:** To determine whether attending a BRCA group influences the choice for surveillance or prophylactic mastectomy.

**Patients and methods:** 196 BRCA1/2 mutation carriers were included of who 89 participated in a BRCA group. Preference for prophylactic mastectomy was registered after mutation carriership was revealed, thus before first attendance of the group.

**Results:** Characteristics of patients, who did or did not participate in a BRCA group, did not show any difference in demographic variables, age at breast cancer diagnosis, menopausal status or family cancer history. Preference for preventive mastectomy or surveillance was not significantly different in participants and non-participants, being 31/89 (35%) and 27/107 (25%) respectively ( $p=0.13$ ).

After a median observation period of 2 years (range 1-9 years) the percentage of women actually performing preventive mastectomy was significantly higher in participants than in non-participants of a BRCA group, 45% and 29% respectively ( $p=0.02$ ). However, in the group with prior preference for mastectomy who did and did not participate in a BRCA group, prophylactic mastectomy was performed in 90% and 55%, respectively ( $p=0.003$ ), in the group with prior preference for surveillance these percentages were 19% and 20% respectively ( $p=0.09$ ).

**Conclusion:** The impact of a BRCA support group on the choice for prophylactic mastectomy is limited, and is determined by the woman's preference prior to the establishment of a BRCA mutation.

##### EP11.2

##### Development of a tailored, online decision aid on screening options for unaffected men with a family history of prostate cancer

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**PURPOSE:** Men at increased risk for prostate cancer on the basis of family history are confronted with difficult decisions regarding the management of that risk. The information that needs to be conveyed is complex, and men often have difficulty accurately weighing up the costs and benefits of screening tests such as prostate specific antigen (PSA) screening.

**METHODS:** This study has two stages: (i) to develop and pilot-test an online, tailored decision aid for unaffected men with a family history of prostate cancer to inform them about their risk management options; and (ii) to compare in a randomised trial the efficacy of the decision aid to that of a comparison website amongst men at increased risk of developing prostate cancer on the basis of family history.

**RESULTS:** The early prototypes of the online decision aid were developed using an iterative process involving a working party comprised of experts and a consumer representative. It provides information on the genetics of hereditary prostate cancer, personal and family risk of developing prostate cancer; putative protective factors; screening tests and efficacy and side-effects of treatment options. Pilot-testing with approximately 20 unaffected relatives of men diagnosed with prostate cancer is currently underway, and results will be reported from the pilot testing phase.

**CONCLUSION:** A decision aid seems particularly suitable to provide

this group of men with decision support, and we anticipate that men will report better understanding of different risk management strategies and increased consumer satisfaction and will be more involved in decision-making.

## EP12. Family dynamics and genetic conditions

### EP12.1

#### Perceived impact of BRCA1/2 testing on familial relationships and association with psychological distress

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**Objectives:** This study describes the perceived impact of BRCA1/2 testing on relationships with first degree relatives. It also assesses whether experiencing a negative impact on relationships is associated with psychological distress and whether a positive impact is protective against distress.

**Methods:** A total of 636 French-Canadian women from 236 families, who underwent BRCA1/2 testing in the context of the INHERIT BRCAs program between August 1998 and July 2004, were enrolled. Among them, 44% had already had cancer; 131 women were found to be mutation carriers, 172 non-carriers, and 333 had an inconclusive result. Questionnaires completed at pre-test and then at 1, 12, and 36 months post-disclosure were used to assess the perceived impact of testing on family relationships and psychological distress.

**Results:** Three years post-disclosure, 11% of women perceived that testing has a positive impact on their familial relationship compared to only 3% who perceived that the impact was negative. Among mutation carriers, these proportions reach 16% and 7%, respectively. After controlling for a range of socio-demographic, medical, and psychosocial factors, experiencing a negative impact on relationships with a first-degree relative was significantly associated with long-term distress ( $p=0.04$ ). However, the perception that genetic testing had a positive impact on relationships was not protective against distress.

**Conclusions:** Although the perception that genetic testing had a negative impact on family relationships is associated with long-term distress, this is quite infrequent. More research is needed to better understand how the impact of BRCA1/2 testing on family dynamics may affect individuals' quality of life.

### EP12.2

#### Social Intervention in the context of presymptomatic testing for late-onset neurological disorders

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These diseases have several social and psychological implications (structural, processing, cognitive, emotional changes). Social intervention should be based in the systemic concept: good balance between psychodynamics demands of illness and family dynamics and their resources is determinant for the adaptation process and confrontation with the illness.

In our protocol of presymptomatic testing, the social worker assesses two groups of risk factors: (1) vulnerability centred on the subject (the genetic risk, personality resources, cognitive resources, etc.); and (2) vulnerability connected with inadequacy of the environment (familial structure, separation or death of a parent, chronic disease, economical fragility/poverty, social isolation, etc.).

Social Intervention is carried out through (1) exchanges holding emotional positive attitudes; (2) (formal or informal) counselling, allowing the establishment of interactions that have the aim of sharing information on the disease; (3) material or instrumental support; (4) technical support or of services; and (5) access to new contacts. In terms of social intervention, several people have also been helped with waivers for fees in health care (exemption from fees, medication, technical aids) (23); in the process of their incapacity retirement (10), professional conversion or changes (5), in the process for fiscal (IRS) benefits (3), and help finding a nursing home, continued care unit or care at home (6).

Social intervention, in presymptomatic testing for late-onset, so far incurable, neurological diseases, has the aim to help the persons at-risk and their family to keep balance and functioning, when confronted with

crises, such as test results or beginning of symptoms.

### EP12.3

#### Susceptibility to type 2 diabetes: perceptions and family communication regarding inheritance and primary prevention

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Type 2 diabetes is a multi factorial disease, which means diabetes onset is triggered by interaction of (multiple) genes and environmental factors. Family history may serve as a good predictor of diabetes risk. People at risk can delay or possibly prevent diabetes by following a healthy lifestyle. We wonder whether this information is known by patients and whether they (are able or willing to) share it with their relatives.

544 People diagnosed with type 2 diabetes filled in a questionnaire. It appears in most families talking about diabetes is not taboo. Most respondents indicate that 'sometimes' diabetes is discussed; mostly initiated by the patients themselves. Subjects include mainly diabetes related problems; less developing or preventing diabetes onset.

Approximately half of the respondents knew about the inheritable character of diabetes; half of the group prefers more information provided by professionals. A majority agrees on the potential role of patients informing their family; half of the group actually intends to do so. They indicated they knew what, how, and whom to tell. Approximately half of the respondents believes patients should be coached by health professionals. In the presentation, relations between demographic variables (including ethnicity), diabetes related factors (including illness perceptions and family history), knowledge on inheritance and primary prevention, and family communication will be outlined.

In our opinion, the results may contribute to more targeted and effective health promotion strategies in diabetes prevention programs. However, we experienced quite different reactions, mostly in non-responders, discussing health issues in relation to family matters.

## EP13. Living with genetic disease

### EP13.1

#### The long-term information and support needs of BRCA1/2 mutation carriers

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Genetic testing for inherited breast/ovarian cancer can have far reaching implications, both for the individual and their family. Research to date has mainly focused on the short-term impact of testing. The aims of this study were to explore the long-term impact of being identified as a BRCA1/2 mutation carrier, investigate the information and support needs of this group and explore best how cancer genetics services can meet these needs.

Data was gathered using semi-structured interviews with women who had undergone genetic testing in Wales and had received a mutation positive result. The interview transcripts were thematically analysed to gain insight into participant's thoughts and experiences.

For these women their 'genetic journey' did not end upon receipt of their test results. The findings suggest that BRCA mutation carriers may have specific needs for information and support that extend beyond the genetic testing process and that these needs may vary over time. A number of real or perceived barriers were identified which prevented participants from accessing support and information. Participants made valuable suggestions as to how cancer genetics services could address these issues.

It is likely that no single intervention will be effective in addressing the needs of this heterogeneous group. Further large scale research is needed to investigate how best to target resources to meet client needs. This study explores the views of this group, highlighting issues that are important to them. The findings emphasise the fact that long-term user involvement is essential in the planning and optimising of service provision.

**EP13.2****Decision making dilemmas for E-cadherin mutation carriers: A family case study****S. C. Downing:***Department of Medical Genetics, Addenbrookes Hospital, Cambridge, United Kingdom.*

This case study presents two siblings, whose health beliefs, career and family responsibility had a strong influence on how they perceived their risk, management choices and decisions. Both were carriers of an E-cadherin (CDH1) mutation and chose to manage their risks differently. Gastric cancer affects 15 per 100,000 people in the UK and around 3% arise from a clearly identified inherited gastric cancer predisposition. One third of families with a strong history of diffuse gastric cancer have germ line mutations in the E-cadherin gene. Mutation carriers have a 70-80% life time risk of developing gastric cancer with associated poor outcome, they also have an increased risk of lobular breast cancer and colon cancer. Predictive testing is available for individuals where a familial mutation has been identified. A positive result provides challenging decisions regarding personal risk management with the option of endoscopic screening or prophylactic gastrectomy. Screening is unproven and there is concern that early cancer may be missed since it is submucosal. The alternative option of surgery carries a high morbidity with life long adaptations required; limited data exists on the outcome for prophylactic gastrectomy. One sibling opted for preventative surgery, the other endoscopy screening. Counselling issues included: career changes, family dynamics, psychosocial aspects, coping with major surgery, and quality of life. Management of cancer risk is a personal issue, risk management options should be explored in depth to enable the individual to make fully informed decisions.

**EP13.3****Quality of life in adults with familial adenomatous polyposis (FAP)****K. Fritzell<sup>1</sup>, J. Björk<sup>1</sup>, M. Arman<sup>2</sup>, R. Hultcrantz<sup>1</sup>, L. Wettergren<sup>2</sup>:**<sup>1</sup>*The Swedish Polyposis Registry, Stockholm, Sweden, <sup>2</sup>Department of Neurobiology, Care Sciences and Society, Karolinska Institutet, Stockholm, Sweden.*

**Background:** FAP is a disease which among other symptoms is manifested as multiple polyposis with adenomas in the large intestine, rectum and duodenum. These adenomas are very likely to turn cancerous over time. The disease is inherited autosomal dominant. Surgery is the only treatment that can prevent colorectal cancer and prophylactic surgery is performed on all patients. Preoperative symptoms are rare, but a large number of patients report functional impairments after surgery.

**Aim:** The aim of the study was to describe how adult patients with FAP perceive that the disease has affected their life.

**Method:** Adult patients ( $\geq 20$  years) attending the outpatient clinic at The Swedish Polyposis Registry were invited to participate in focus group interviews ( $n=43$ ). Three focus group interviews were conducted. Data was analysed by means of content analysis.

**Results:** The content analysis revealed nine themes: Worries and concerns, Social life, Choice of career and education, Risk of giving the disease to the children, Changed food and toilet habits, Experiences of endoscopic examinations, Relationships to health care providers, Stories about hospital stay at their first surgery, Knowledge of FAP.

**Conclusion:** The study has highlighted several issues of concerns related to FAP. Worries about getting worse due to the disease were expressed by many of the participants but not anyone mentioned cancer as a threat for future health. Due to the heredity of the disease, having own children or not was a deep and complex question and the discussion gave an impression of a need to defend one's standpoint.

**EP13.4****Participation in Huntington's disease research:hoping, coping and a nice day out****J. Needs:***Cardiff University, Cardiff, United Kingdom.*

This poster is based on an ongoing PhD examining Huntington's disease (HD) as a case study of how the development of genetic technologies and identification of human genetic mutations, affect the work of the clinic and the lives of patients. Ethnographic data from neurological research clinics ( $n=11$ ) will be used to explore the current nature and structure of clinical work itself, and will illustrate how different mean-

ings of the clinic are enacted by the participants, including patients, family members, carers, and researchers. This work will be used to inform later stages of the PhD, which will include interviews with patients and clinical teams and the examination of an archive of HD patient records from the 1970's to the present day.

Current clinical practice emphasises genetic, biological, cognitive and physical measurement. These are areas associated with neuroscientific research programmes, and patient recruitment is crucial in order to provide large data sets for possible future clinical trials. These data sets will be kept indefinitely and provide material for international collaborative research. However, for patients and families, research involvement is not always the focus. Rather, they view participation as a privileged medical appointment and a way of getting answers to their own situations. They hope for effective treatment via research (stem cells) and participation may provide one strategy for coping with the effects of the disease. The research environment and relationships with researchers at repeated clinic visits also provides familiarity and a social aspect to the whole process, in effect, a nice day out.

**EP13.5****Consensus document on best care in HD: a training/educational program for Italian neurologists****G. Jacopini<sup>1</sup>, P. Zinzi<sup>1</sup>, A. R. Bentivoglio<sup>2</sup>, M. Frontali<sup>3</sup>:**<sup>1</sup>*ISTC/National Research Council, Rome, Italy, <sup>2</sup>Istituto di Neurologia Università Cattolica del Sacro Cuore, Policlinico "A.Gemelli", Rome, Italy, <sup>3</sup>INMM/National Research Council, Rome, Italy.*

Huntington's disease is a rare, complex, hereditary disorder, characterized by motor as well as psychiatric symptoms; very often, neurologists are unable to handle these patients, lacking guidelines about the most appropriate way of care for them. In July 2007, upon request of the Italian Association for Huntington's Chorea (A.I.C.H-Rome and A.I.C.H-Milan), our research group organized a multidisciplinary panel of experts with the main task of providing recommendations for the best care in HD. The panel produced a "consensus document" with recommendations which address HD patients and families' main needs, combining together scientific information and long lasting clinical experience. The document covers multiple aspects: making the diagnosis and communicating it, therapeutic management and paramedical treatments, nutrition and dietary supplements, genetic risk and genetic testing protocol, co-morbidity, palliative and end-of-life care, non medical support, indications for referral.

The document, written in a synthetic style, contains practical recommendations aimed at minimizing inappropriate care and introducing research findings into clinical practice of neurologists not familiar with HD issues. The document will be printed (10,000 copies) and, starting May 2008, first introduced in the course of three main educational events (in Northern, Central and Southern Italy) for neurologists. Then the document will be delivered to 3,500 neurologists working both in public health care settings and in the private field. In the future we plan to assess the impact of the document through meaningful and measurable outcomes.

**EP13.6****Living with a person with Huntington's disease****A. J. A. G. Van Tongerloo, A. M. J. J. De Paepe:***University Hospital Gent, Gent, Belgium.*

**Introduction:** Huntington's disease, an autosomal dominant neurodegenerative disorder, is characterized by a triad of progressive motor, cognitive and emotional symptoms. In 1994, predictive testing through the direct DNA test became available. The psychological impact of carriership on the testee and his/her partner has been extensively evaluated and reviewed. Few studies however, have been conducted to explore how living with a person with the disease affects the healthy partner's daily life.

**Materials and Methods:** In January 2008, a qualitative study was set up, in which partners of persons with HD are questioned through a semi-structured interview. Topics that are questioned are: coping with the partner's symptoms, the symptoms that are the most difficult to deal with, the changes in personal life of the healthy partner, and the future perspectives for the healthy partners. Furthermore, participants are asked about their feelings towards their partner, and about communication on the disease with the affected partner, with their children and with significant others.

**Results:** Preliminary results of this study, which is still ongoing, will be presented. It is anticipated from our clinical experience that emotional/character changes of the partner are most difficult to cope with, since the partner often becomes a "different" person to live with. Changes in personal life include mainly changes in the role model in the partner relationship. Healthy partners often experience the daily care and the material consequences of Huntington's disease as a major burden for the future.

#### EP13.7

#### **Psychosocial aspects of living with Long QT Syndrome. A qualitative study**

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**Background:** Long QT syndrome (LQTS) is a genetic disorder characterized by prolongation of the QT interval on the electrocardiogram, potentially leading to life-threatening arrhythmias and increased risk of sudden death. In Norway LQTS patients and their families are offered diagnostic or predictive genetic testing. Norwegian legislation states that genetic counseling is mandatory in connection with genetic testing of healthy individuals.

**Purpose:** The purpose of this qualitative study was to

- Investigate the psychosocial aspects of living with LQTS
- Describe LQTS patients' experiences with healthcare services.

**Material and methods:** In-depth interviews with seven Norwegian adults tested for long QT genetic mutations, was conducted. Four participants had an implantable cardiac defibrillator (ICD).

**Results:** Participants experienced worries and limitations in daily life and it was a general wish among them to be able to talk to someone with the same condition. Their main concern was not for their own health but for their children or grandchildren. Having an ICD was experienced as an additional safety but it could also cause social embarrassment and anxiety. Early and gradually acquired knowledge of the syndrome was reported as an advantage. Healthcare providers' minimal knowledge of LQTS resulted in uncertainty, misinformation, and even wrong advice on treatment.

**Conclusion:** Knowledge of the challenges met by this patient group is important in order to provide adequate genetic counseling. Further investigations into the psychosocial aspects of LQTS are desirable.

#### EP13.8

#### **Unmet information and support needs amongst individuals affected by one of two rare cancer syndromes: MEN2A and VHL**

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Multiple endocrine neoplasia type 2A (MEN2A) and von Hippel Lindau syndrome (VHL) are rare, inherited, early onset cancer syndromes characterised by the development of tumours in one or more parts of the body. Genetic testing is typically carried out in young children with a family history of either condition. Individuals identified as gene carriers undergo lifelong surveillance in order to detect tumours at an early stage. Little is known, however, about the psychosocial experiences of individuals affected by MEN2A or VHL. This study aims to identify the unmet information and support needs of individuals affected by these rare cancer syndromes. Patients with a confirmed diagnosis of MEN2A or VHL were ascertained via the Hereditary Cancer Clinic at Prince of Wales Hospital, NSW, Australia. A semi-structured individual interview was developed to assess five broad themes: impact of MEN2A/VHL on various life domains such as relationships, education and employment; attitudes towards childbearing; coping strategies; family communication about the disease; and unmet information and support needs. Interviews with approximately 20 patients and 16 caregivers are currently underway, with 16 completed patient interviews and 8 completed caregiver interviews. Data will be analysed separately for patients and caregivers, using qualitative data analysis software, QSR N6. Partic-

ular focus will be placed on potential thematic differences between cancer groups and genders. These data will inform the development of a survey instrument, which we aim to administer on an international scale through collaborations with the Association for Multiple Endocrine Neoplasia Disorders (AMEND) and the VHL Family Alliance.

#### EP13.9

#### **Managing relationship needs while living with chronic genetic illness**

F. Pearce;

*Genetic Health, Parkville, Victoria, Australia.*

The special care requirements of children, who progressively become physically and intellectually disabled due to metabolic illness, can be emotionally and physically overwhelming.

All family relationships are under pressure in these circumstances. At the same time, people discover capabilities and strengths that would rarely be known about or used, under more normal situations. Often, the sick child's primary care-giver (usually their mother) has to make choices from several competing needs at the one time. How are these choices made? On what basis are they prioritised? A great number of relationships fail in these circumstances, however others are awe inspiring.

This presentation will include case material that demonstrates how some couples cope and how others feel forced to 'give up'.

#### **EP14. Other relevant psychological and social topics in genetics**

##### EP14.01

#### **Mental representations of pregnant women with advanced maternal age (AMA) who undergo amniocentesis: a unique dynamic process**

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Non routine medical procedures, such as amniocentesis, have been suggested to have an emotional impact on pregnant women. Anxiety is an obvious consequence, the psychological "suspension" of the pregnancy might be another one. We propose that the process of accepting the reality of gestation and the reality of the foetus, expected to occur respectively in the first and second trimester, is postponed in AMA women who undergo amniocentesis until after the result comes through.

Building mental representations about the foetus is an important part of the psychological process that takes place during pregnancy. In fact, some studies have questioned the importance of these representations for the quality of the mother-baby relationship/ interaction. Amniocentesis is thus performed during a crucial period of intense and dynamic internal activity. The purpose of this study was to understand how does this procedure influence the dynamic process by which AMA women build representations of the foetus as their "child-to-be".

We applied an adaptation of Interview R to a group of 35 pregnant AMA women (A) and a control group (NA), before and after amniocentesis. Before amniocentesis, group A gave "less rich" and "less positive" descriptions of their future babies, and characterised them as "less active" and "quieter". After the results were known, the only difference between the two groups was that NA women continued to describe their future children in a more positive way.

These findings suggest that the dynamic process of maternal mental representations is unique in AMA women who undergo amniocentesis.

##### EP14.02

#### **GeneBanC. Studying the ethical, legal and social aspects of Biobanking**

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**Background.** The last few years have witnessed an important expansion of collection and processing of human biological samples and of the related information data. Biobanks are huge repositories of human biological specimens and have a strategic importance for genetic research, clinical care and future treatments.

**Objective.** GeneBanC is an acronym for the research project *Genetic bio- and dataBanking: Confidentiality and protection of data. Towards a European harmonization and policy*. This E.U.-funded research project that has been funded by the European Commission and aims to investigate the ethical, legal and social issues of three types of biobanks: classical banking, population banking and forensic DNA databases.

**Method.** The workload has been split up in various workpackages, studying respectively (a) the issue of privacy and confidentiality in biobanking; (b) the existing regulatory framework of biobanks across the E.U. and the regulation regarding the establishment, management and functioning of biobanks; (c) forensic genetic databases; (d) governance aspects of biobanks.

**Expected results.** It is expected that the results obtained within the different objectives described above will be of great use for the development of policy oriented recommendations concerning the organisation and management of biobanks. Proposals will be elaborated in order to reach where appropriate a harmonized regulatory framework across the European Union.

#### EP14.03

##### Support groups for women carrying a *BRCA1* or *BRCA2* mutation : a first experience.

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During consultations we felt that women carrying a *BRCA1* or *BRCA2* mutation had a strong desire to share their experience. We decided to set up a support group for them.

The aim of this support group was to give the patients a place where each individual could be heard, and where experiences and information could be shared.

53 women patients were invited by mail, and 8 of them decided to participate. They all had a personal history of breast and/or ovarian cancer. Six had undergone preventive surgery.

We chose a non-directive method, allowing the patients to decide what topic was to be addressed at each meeting. We met every 1.5 month, during 1 hour, for 7 months (5 meetings). Meetings were led by a psychologist, a geneticist and a clinical research assistant. The themes addressed were: cancer (family history, isolation, relationships with closed ones, the body image, treatment, surgery, femininity), loss (of self, of a part of one-self, of a loved one), pre-symptom analysis, maternity, preventive surgery, and medical questions.

Our goal was met. Indeed, a positive dynamic was present throughout the meetings. Most of the themes of concern in this particular patient population were addressed. The patients' feedback was very positive. It allowed some of them who could not talk at home to share their difficulties, and others to begin a dialog with their closed ones.

This year, we started 3 new groups, involving 22 patients, based on Spiegel's supportive-expressive method.

#### EP14.04

##### Dying with a family history of cancer: A phenomenological study

**A. K. Lillie;**

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There has been widespread media coverage about the potential for an inherited predisposition to cancer in Britain. This study aimed to describe how this was affecting care needs of palliative care patients. Data was collected through recorded, semi-structured interviews with twelve patients with advanced cancer. Purposive sampling ensured that participants had been predeceased by at least one first degree relative with cancer. Data was coded using an iterative approach into themes that arose from the participants experiences. Van Manen's (1990) schema of existential analysis was then used to identify the relationship between the themes under investigation.

This study showed that patients were dying with concerns about the implications of their disease for their families. Concerns for children were widespread and existed in patients who did not think that their own cancer was associated with an inherited susceptibility, patients who had been informed that they were not at high risk of familial disease and in patients who had not discussed their anxieties before.

The participants' experience of dying was affected by previous death within the family: especially by the deaths of relatives who had died at a younger age than normal. It changed the way the participants com-

municated about cancer and affected family cohesion and hence the support available to participants. It also increased their awareness of their dying trajectory, altering the way they coped with the disease. This study show that there are complex challenges for health care professionals when supporting dying patients worried about their family history of cancer.

#### EP14.05

##### Translating research into practice: Health care professionals' and researchers' understanding of cancer genetics activities in the UK

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DNA-testing for hereditary cancers and high risk cancer surveillance in the UK takes place either as part of research protocols (and thus, requires ethical approval) or is offered as an NHS clinical service. The route chosen may depend on a range of factors. This multidisciplinary project investigates healthcare professionals', patients' and regulators/other stakeholders' understandings of cancer genetics activities within the UK. It looks at how these different groups conceive of research and clinical practice, and aims to identify any perceived ambiguities and practical and/or ethical problems that are generated by these activities for the different actors. Semi-structured interviews have been carried out with healthcare professionals and researchers (HCPs) (n= 40) who are involved in cancer genetics research and/or provide a clinical cancer genetics service. The interviews suggest that research and clinical practice are perceived as standing in a complex relationship with each other. In some cases our respondents made strenuous efforts to distinguish these activities, while at other points in their interview they struggled to identify differences between them. The boundary between research and clinical practice within cancer genetics, could thus, be characterised as ambiguous, flexible, permeable and a permanently shifting phenomenon. The strategies that healthcare professionals use to distinguish these activities are described.

#### EP14.06

##### Evaluating the impact of a self-help coping intervention: a study outline of the CARIAD Trial.

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**Background** Around a third of individuals undergoing genetic risk assessment report high levels of distress and are likely to benefit from increased psychological support. A self-help leaflet has been developed, based upon coping theory, to help individuals cope with unwanted worries whilst waiting for genetic information. The intervention encourages individuals to restrict consideration of the issues related to their risk for a set time each day, and to actively distract from intrusive thoughts at all other times. The CARIAD trial is evaluating the psychological impact of this intervention.

**Study aims** 1) To evaluate the short- and longer-term impact of the intervention on levels of distress at key stages of the assessment process 2) To explore the impact of the intervention on emotional responses and coping.

**Design** Recruitment commenced in September 2007. Over a nine month period 1,000 male and female referrals into the Cancer Genetics Service for Wales will be pre-randomised to receive either the coping leaflet or standard care and asked to complete a postal questionnaire at 3 time points: upon referral, whilst waiting for genetic risk information (4 weeks post-referral) and one month post-risk.

**Outcomes** The primary outcome measure is the level of situation-specific distress over time, as measured by the Impact of Event Scale. Secondary outcomes and potential moderators include positive and negative affect, risk understanding, illness perceptions, and coping. The intervention may also be appropriate during other periods of waiting and uncertainty such as undergoing predictive or prenatal genetic testing or awaiting screening outcomes.

**EP14.07****Psychosocial and demographic profile of a deaf community in the Altai Republic: a pilot study****O. L. Posukh, O. V. Posukh;***Institute of Cytology and Genetics, Novosibirsk, Russian Federation.*

The study is based on data obtained from self-completion questionnaires designed to evaluate marital status and marriage patterns of deaf persons, their communication mode, access to education, employment and information, and their living standard and social status. The participants belonged to the association of deaf and hard-of-hearing people in the Altai Republic (south Siberia, Russia).

In recent years total number of the association members varied from 150 to 160 people at the age of 18-85 years with approximately equal gender ratio. Most of them have congenital or early onset severe-profound deafness. About two thirds of adult members are married to deaf partner and almost all the young unmarried people with hearing loss tend to prefer deaf partner in future. Deaf participants indicated sign language as communication mode with other deaf persons, whereas in communication with hearing people they use lip-reading, writing, and oral language skills, if any. Their embarrassment in contacts with hearing people due to mutual misunderstanding leads to preferential contacts with deaf people. Most of participants consider their hearing loss as disability which limits access to education and employment, and decreases their social status and living standard.

Our molecular-genetic studies revealed deafness caused by Cx26-mutations in not less than 15% of examined members of this association, and several families with hereditary deafness of yet unknown etiology.

This study has implications for genetic counselling for families with hereditary deafness, and provides insight to deafness prevalence pattern in the Altai Republic.

Work is supported by Russian Foundation for Humanities (07-06-00765a).

**EP14.08****Psychosocial impacts of the neonatal hearing screening**

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Since February 2005, an experimental program of neonatal screening of the congenital deafness is in process in France.

Our purpose is: - to estimate the psychological impacts of the screening; - to measure the risk of creating an anxiety in the parents and thus to hinder the development of the attachment mother / child links, which set up in the very early neonatal period; by the study of the false positive families (hearing children with pathologic automatic ABRs).

A questionnaire was realized, and sent by mail to families, accompanied with a letter explaining the reasons of our research. The first part of the questionnaire concerns the information which was given to the parents before the screening, the result obtained at the test and the information which followed this result. The second part of the questionnaire focuses in the way back at home looking forward to the consultation with the ENT, in which the hearing defect will be confirmed or not. The third part of the questionnaire concerns the feelings of the parents at the time of the ENT consultation, the information given to them, and the impact of the final diagnosis. Then the last part is about the general feelings of the parents as regards the neonatal screening of the deafness and finally the impact of this screening on their relations with their baby.

This work allows us to give some recommendations to improve this screening in the future.

**EP14.09****Exploring the impact of genetic testing for Familial Hypercholesterolaemia: a family perspective.****M. S. Watson<sup>1</sup>, D. Townsend<sup>2</sup>, I. McDowell<sup>1</sup>, K. Featherstone<sup>3</sup>;**

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Familial Hypercholesterolaemia (FH) is an autosomal dominant disorder with a prevalence of 1 in 500, approximately 110,000 people are

thought to be affected in the UK, but unfortunately the majority remain undiagnosed. Effective preventative treatment is available, if untreated it leads to premature Coronary Heart Disease (CHD) and death. Cascade testing using genotyping has recently become available in South Wales on a research basis.

The main aims of this study were to explore how patients and their family members receive, make sense of and transmit genetic information and the impact this dynamic process has on perceptions of risk. Face to face semi structured interviews were conducted with seven patients in whom a clinical diagnosis of FH had been made and who had recently undergone genetic testing and received a mutation positive result. A further seven interviews were conducted with members of their families who had also undergone genetic testing to follow the flow of this genetic risk information. The interview transcripts were thematically analysed to gain insight into their experiences.

The findings suggest that this genetic information help the patients to make sense of their condition. Family members reported an open style of communication although this process and emotional responses to genetic risk information were complex. This research highlights some key issues for future research of this complex dynamic process.

**EP14.10****Carriers of a cancer pre-disposition gene: Gaining an insight of their ongoing needs**

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Research has shown that people who carry a cancer predisposition gene (gene carriers) value follow up from a Familial Cancer Centre (FCC) after learning of their results. Gene carriers may require ongoing support over time, as their needs in adjusting to their genetic status may change through the family life cycle. This ongoing support may include a discussion of issues around impact and adjustment to the genetic test result. From an FCC perspective, communication of genetic information to family members also needs to be explored.

The follow up practice at the Royal Melbourne Hospital Familial Cancer Centre has involved a yearly phone call from a Genetic Counsellor to a gene carrier. This format may not be optimal for many gene carriers. As the number of gene carriers increase, many FCCs may find their increased workload unsustainable.

This presentation will summarise a trial of an alternative strategy of supporting gene carriers over time. In addition, issues identified by the gene carriers themselves will be described.

**EP14.11****Comparison of experiences between Italian and other European Genetic Nurses**

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The professional figure of the Genetic Nurse (GN) is well defined in some European and extra-European Nations. In these countries there are formal courses of studies, which allow the students to graduate to GN.

In Italy there isn't either a formal acknowledgement of the figure of the GN, nor does exist a specific education for non-medical personal working in clinical genetic institutes.

Nevertheless we knew that some people, scattered among Italy and generally without knowing of the existence of other colleagues sharing the same mansions, were working in the field of clinical genetics.

With the aim of creating a national net of GNs and of promoting the official situation of this figure after specific learning programmes, we made the first census of the Italian GNs.

After phone contact with the directors of the centers for clinical genetics, we submitted a questionnaire to the people working as GNs and analyzed the results.

The activities of the GNs in other countries were taken from literature and through direct contact with Dr. Heather Skilton.

Our data showed marked heterogeneity of duties of the GNs in Italy but the majority of the mansions matches that of the foreign colleagues. The Italian GNs feel in need of a specific course of studies to prepare them to a highly specialized work, which should be acknowledged by the national health system.

**EP14.12****The role of the specialist midwife in Fetal Medicine Units.**

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The role of the midwife in fetal medicine unit in UK is quite well defined. Some specific actions are: advocate and support for women; provision of information about the nature of the abnormality (both written and verbal) for women and other health professionals; counselling women about their choices regarding the pregnancy, and preparing women for either termination of pregnancy or continuing with a baby who has abnormalities; led clinics for antenatal counselling and follow up after TOP and bereavement care; communication with other health professionals to ensure continuity of care for women. In Italy the role of specialist midwife is still quite heterogeneous, depending on the presence or not of a well defined Fetal Medicine Units. We present a collaborative effort to share experience between UK and Italian midwife in defining the appropriate skill required as advanced communication skills; counselling training; specialist knowledge about prenatal diagnosis; highly developed analytic and judgemental skills; planning and organisational skills; technically adept; leadership skills; IT literate; support for self- emotional and psychological.

The aim of this collaborative work is to define a general framework that describe the role of the specialist midwife in fetal medicine in order to establish a model that can be applied internationally.

The work of the specialist midwife in Fetal Medicine is challenging and rewarding, and offers scope for providing excellence in clinical care for pregnant women and their families.

**EP14.13****Collecting data of HD patients for the Euro-HD Registry: an update from Rome sites**

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The Euro-HD Network is aimed at providing a platform for professionals and people affected by HD and their relatives in order to facilitate collaboration throughout Europe. The core of the study is the Registry, a systematic collection of clinical research data of HD patients, of mutation carriers and of individuals who are part of HD families.

Two study sites participate to Euro-HD network in Rome: one is at the Consiglio Nazionale Ricerche (C.N.R.) and is based on the long lasting cooperation between two main institutes of public research, the Institute of Cognitive Sciences and Technologies (I.S.T.C/CNR) and the Institute of Neurobiology and Molecular Medicine (I.N.M.M/CNR); the other one is at the Neurology Department of the Università Cattolica del Sacro Cuore (U.C.S.C.) Both sites are involved in running an outpatient clinic for HD patients started in 1989 as a common research project and which has assumed, since 1994, the characteristic of a multidisciplinary clinic. The individuals followed at the clinic are 312: 65% are symptomatic patients, 20% are subjects at risk (pre-test neurological examination), 15% are asymptomatic gene carriers (post-test follow-up).

For the Euro-HD project, from Sept 2004 to Dec. 2007 we have completed and entered into the Registry the data collection of 75 patients (37 Males and 38 Females): 69 symptomatic patients and 6 asymptomatic gene carriers. For further 15 patients we are now completing the last forms and their data will be entered quite soon.

Data analysis and comments on different aspects of data collection will be reported.

**EP14.14****The risks & benefits of screening for Klinefelter syndrome: A critical analysis**

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**Background:** Klinefelter Syndrome (KS) is a genetic condition (47,XXY) affecting males and resulting in a spectrum of clinical features, ranging from infertility, androgen deficiency and breast development to varying levels of cognitive, social, behavioural and learning difficulties. The prevalence has been estimated to be 1:650, yet up to 70% of males remain undiagnosed. Early identification has been advocated for many years, but population-based genetic screening for KS has never been explored.

**Aim:** To identify the potential risks and benefits that could arise from implementing population-based genetic screening for KS at different ages and stages of development.

**Method:** A framework was developed to assess the medical (hormone, therapeutic interventions), psychological (self-esteem, relationships) and ethical (autonomy, associated stigma) implications of genetic screening for KS in four hypothetical age-dependent scenarios - newborn, infancy, primary school entry and high school entry - chosen as providing opportunistic circumstances in which an individual might be evaluated. The outcomes have been considered in relation to diagnosis in adulthood and the most common scenario of lifelong non-diagnosis. Evidence of potential risks and benefits associated with KS diagnosis was collected by analysis of the existing literature.

**Results:** Our analysis indicates that while there is information on available medical, educational and psychological interventions, there has been no consideration of the impact of age of diagnosis and the related timing of interventions on psychosocial and other quality of life outcomes.

**Conclusion:** More research is needed to fill these evidential gaps and inform decisions regarding population-based genetic screening for KS.

**EP14.15****Genetic counselling training and supervision in a second language: is it different?**

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**Purpose:** Live supervision is a key component of the training of genetic counsellors. However, the ways in which training and supervision differ for students and supervisors when these activities occur in a second language have not yet been explored.

**Method:** A questionnaire was distributed to members of the CAGC and NSGC using the online survey provider, [www.surveymonkey.com](http://www.surveymonkey.com). Data were analyzed using a consensual qualitative method modified from Hill et al. (1997).

**Results:** Supervisors find it difficult to assess students' psychosocial counselling skills in the second language, and feel personal discomfort in having an incomplete understanding of session content. Students, in turn, describe decreased competence when counselling in their second language, and a greater focus on the medical aspects than on the psychosocial dimensions. Interestingly, students whose second language is French (mainly Canadian) describe more negative experiences and more difficulties in certain areas, such as building rapport with patients, than do students who counsel in Spanish (mainly American). This may reflect differences in patient expectations for receiving service in their native tongue in Canada compared to the US. The language of training, whether it is the student's first or second, was described as having a major impact on counselling abilities and comfort level in the second language. Overall, however, the use of second languages in training seems to have a positive impact for both students and supervisors, leading to increased satisfaction in providing linguistically and culturally-centered patient care. The implications of these findings for genetic counselling training will be discussed.

**EP14.16****"Asking for oncogenetic counseling": evaluation of psychological aspects, need for information and perceived risk**

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The amount of clients seeking oncogenetic counselling (OGC) is rapidly growing up. Understanding communicational aspect and clients' styles of information seeking, together with personality, is fundamental

to optimize counseling service. We necessitate to analyze psychosocial features to fit counseling process to client personality and needs. To reach this goal, since 2004 we have evaluated needs for information, motivations and psychosocial characteristics of individuals asking for OGC because of their familial history of common cancers (breast, colon, thyroid, etc.). 110 counselees fulfilled a questionnaire before receiving OGC assessing: sociodemographic features, health locus of control, monitoring/blunting, general self efficacy, motivations, emotional state and perceived risk. 76 were affected by cancer and 34 were unaffected. Cancer risk was assessed either with genetic testing or with validated probabilistic models. Our results have showed that counselees have: no clinical psychological distress, high level of self efficacy but not a specific style of information seeking. They feel at high risk for cancer running in the family and they strongly want information about surveillance and prevention actions. Perceived risk is related to some psychological variables (coping and mood) and in unaffected people it is not related to cancer risk estimated. We can conclude that, individual seeking OGC have adaptive psychological resources that let them cope with familial cancer risk, and that evaluating psychological profile has several implications into clinical practice, because psychological variables significantly influence risk perception.

#### EP14.17

#### Triangulation of methods: a novel approach to investigating valued outcomes from using clinical genetics services

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The patient benefits from using clinical genetics services are hard to measure, and there is little consensus about appropriate outcome measures. This paper describes a programme of research that aimed to work towards a core set of outcome measures suitable for evaluating clinical genetics services. The approach involved 4 types of triangulation (method, investigator, discipline and data). Three methods were used:

(1) A systematic review of the literature identified the validated outcome measures (30 genetics-specific and 37 generic) used, or developed, to evaluate clinical genetics services.

(2) A Delphi survey, aimed at identifying the degree of consensus about the relevance of existing outcome measures, was completed by a panel of 115 genetics clinicians and 72 patients of clinical genetics services. At least 75% of the panel agreed that the following outcome domains were useful: knowledge of the genetic condition, decision-making, perceived personal control, risk perception, satisfaction, meeting of expectations, coping, accuracy of diagnosis and quality of life.

(3) Qualitative research (7 focus groups and 19 interviews) with patients of clinical genetics services, patient representatives and genetics health professionals explored the outcomes valued by those stakeholders, and resulted in construction of a model of empowerment.

Data triangulation clarified that the qualitative findings support many of those from the Delphi, but identified some additional valued outcome domains that are not captured by available outcome measures, such as empowerment of other relatives and future generations. In conclusion, triangulation is a useful approach to investigate complex areas, such as outcome measurement.

#### EP14.18

#### Psychological evaluation of familial ovarian cancer screening (PsyFOCS): Study update and baseline psychological data

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Purpose: The UK Familial Ovarian Cancer Screening Study (UK FOCS) aims to determine the effectiveness of ovarian cancer screen-

ing for women at significantly increased risk of familial ovarian cancer. Phase 2 of the study involves annual transvaginal ultrasound scan (TVS) of the ovaries and 4-monthly CA125 blood tests with further tests prompted by rising CA125 or abnormal TVS.

Method: PsyFOCS is a prospective cohort study which aims to determine the psychological effects of ovarian cancer screening. Around 66% of women invited to PsyFOCS returned pre-screening baseline questionnaires.

Results: Baseline analyses ( $N=991$ ) showed that clinical levels of depression and anxiety were reported by 2.63% ( $n=26$ ) and 14.63% ( $n=145$ ) of women respectively. However, over a third ( $n=369$ , 37.24%) were moderately or highly distressed about their ovarian cancer risk according to the Impact of Event Scale (IES). There were no significant associations between IES distress and prior ovarian TVS ( $p=.50$ ) or the number ( $p=.95$ ) or reported stressfulness ( $p=.56$ ) of repeat ovarian cancer screening in the past. However, highly distressed women were significantly younger than those reporting moderate or low distress ( $p<.001$ ) and there was a significant association between distress and past ovarian cancer screening recall ( $p<.05$ ).

Conclusion: The results suggest that although most women reported low levels of distress prior to UK FOCS screening, younger women and those recalled for repeat testing may experience greater distress.

#### EP14.19

#### Mental and psychomotor development of 2-year-old PGD/PGS twins compared to ICSI twins

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Introduction: Embryo biopsy is a invasive procedure applied in ART for diagnostic reasons in Preimplantation Genetic Diagnosis (PGD) or to increase pregnancy rate in Preimplantation Genetic screening (PGS). The objective of this ongoing study is to investigate mental and psychomotor developmental outcomes in 2-year-old twins born after PGD/PGS and Intracytoplasmic Sperm Injection (ICSI).

Materials and Method: PGD/ PGS ( $n = 32$ ) and ICSI ( $n = 32$ ) children were recruited from the registry of the Centre for Medical Genetics (UZ Brussel). The obtained response rates were 97% and 94% respectively. All twins were assessed around age 2 and were matched for gender, educational level of the mother, birthorder and mothertongue. There were no significant differences between the 2 conception for these demographics.

Mental and Psychomotor development of the 2 groups of study participants was assessed using the Bayley Scales of Infant Development (BSID-II-NL). Data on interval level were analysed using ANOVA's and a significance level of 0.05 was accepted throughout.

Results: Scores on the Mental Scale revealed no differences between the 2 groups ( $F(2, 62) = 0.09$ ,  $p = 0.764$ ). Scores on the Motor Scale neither showed any differences between the PGD/PGS, ICSI and NC twins ( $F(2, 54) = 1.16$ ,  $p = 0.286$ ).

Conclusion: PGD/PGS twins show similar mental and psychomotor developmental outcomes at age 2, as measured with the BSID-II-NL, in comparison to twins conceived after ICSI. In order to provide more solid reassurance twin children should also be compared to their naturally conceived counterparts.

#### EP14.20

#### Should Skin Color Matter in the Decision to Offer Preconception Genetic Screening?

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Objective: To investigate the influence of U.S. physicians' perceptions of patient race and ethnicity on their decisions to offer preconception genetic screening.

Methods: Primary care physicians ( $N=1,035$ ) were surveyed to study, among other aims, their decisions to offer preconception screening for genetic conditions and the influence of patient skin color and other characteristics on these decisions. Physicians were randomly assigned to view a vignette with a picture of either a "Black" or "White patient".

Results: 978 physicians answered questions on the vignette of a patient considering pregnancy; her race, ethnicity and ancestry were not described. Respondents who viewed the "Black" patient were more likely to offer any genetic screening than those who viewed the "White"

patient (35% vs. 26%, p<.001). Screening for sickle cell disease was offered more often by physicians presented with the "Black" patient (78% vs. 7%, p<.0001) and screening for cystic fibrosis was offered more often by physicians presented with the "White" patient (49% vs. 26%, p<.0001). Physicians who viewed the "Black" patient rated race as more important to their decision to offer screening than those who viewed the "White" patient (76% vs. 49%, p<.0001).

**Conclusion:** As we move beyond screening for single gene disorders to screening for multiple gene variants that predict risk for common complex diseases, a greater understanding of the appropriate use of phenotypic characteristics including skin color and the social constructs of race and ethnicity in decisions to offer genetic services, including preconception genetic carrier screening is needed.

#### EP14.21

#### The emotional experience of termination of pregnancy (TOP): differences between women and men

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Looking at our experience of seven years supporting couples who go through TOP, we propose there is a difference in the way women and men go through the emotional process underlying this experience, from beginning to end.

Once the couple has made the decision to terminate the pregnancy, the medical procedure should take place within a short period, because the interval between the decision and TOP is of intense discomfort. We draw from these women and men own words. Some women said they could no longer stand at the mirror and see their growing belly. Men recalled no longer touching their spouse's belly. Other women said it was shocking to feel the foetus' movements while waiting for TOP.

At the time of TOP, women are confronted with a question: should I see the foetus? Men, on the other hand, know for sure they would choose not to see the foetus because, otherwise, its image would be recorded and they want to forget this experience as soon as possible.

After TOP, women and men usually have different timings when considering another pregnancy. Women take longer to mourn the foetus and men are faster to overcome the fear of another loss.

Understanding the emotional process of TOP makes professionals more able to demystify this painful experience at the eyes of the couples. Studying and accepting differences between women and men going through TOP, helps psychologists minimise the impact of these couples' loss and work with them to find a future project.

#### EP14.22

#### Can a family history questionnaire for children with an undiagnosed genetic syndrome replace a face-to-face appointment with a genetic counsellor?

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Increasing service demands mean that genetics services need to identify alternative ways of collecting family history information other than through face-to-face clinics. This study explored whether a family

history questionnaire developed by the author for parents of children with an undiagnosed genetic syndrome (Wessex Family History Questionnaire, WFHQ) can replace a family history gathering appointment with a Genetic Counsellor and reduce the number of appointments provided. Previous family history questionnaires were evaluated and the following criteria were utilised to evaluate the WFHQ: response rate, degree of omitted data and reliability.

The WFHQ was piloted in a multiphase approach. Seven parents completed the WFHQ and an evaluation questionnaire and the data assessed and compared to the data collected through the face-to-face clinic.

Telephone interviews revealed that the WFHQ was seen as a useful preparatory tool. However parents valued the personal interaction of the clinic appointment, during which Genetic Counsellors were able to clarify family history details and provide reassurance.

The WFHQ clearly enhanced the obtainment of a family history in clinic. However, the use of the WFHQ alone may fail to reduce service demands, as Genetic Counsellors are likely to have to clarify family history details or seek additional details. Further research is required to explore alternative methods of service delivery such as collecting family history data via telephone in order to accommodate rising referral rates.

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#### What Increase the Vulnerability in Children and teenagers with Disability in Rio de Janeiro, Brazil

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This qualitative and quantitative research investigated the scenario of violence against children and teenagers with disability due to genetic conditions, congenital anomalies and related conditions assisted by Deinstitutionalization and Social Rehabilitation Based in Community Program and two Rehabilitation's institution from Francisco de Paula Municipal Foundation (2005-2007). It aimed to make a situational diagnosis of the problem, investigate the kind of violence disabled children and teenagers encounter and, discuss the prevention strategies. The study associated 84 case-studies, using a triangulation method of case-discussions with team work, reflexive groups with families, interview leaderships and participant-observation and, quasi-statistical approach (Becker, 2000). The sample of 33 cases was picked through analysis of the study's protocol considering the variables of most interests to the research. It was observed that family negligence is the most recurrent form of violence; there is a dominion of the male sex case in the minor age and female in the age of 24 years; 88% of the children and teenagers with disability in situations of violence investigated live in Favelas, in houses that lack basic infra-structure. Most families have income inferior to two brazilian minimum wages; there are situations where the family is solely sustained by the governmental benefit received by the disabled child (50%). The social isolation of the families, the absence of the public power, the social impact of the drug traffic and the recurrent basic human rights violation are aggravating factors to the violence risk of those who already have vulnerability due to the disability condition.