PL1.Dissecting the molecular pathology of coeliac disease

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Coeliac disease has become one of the best-understood immune-related disorder. The disease presents in the small intestine and results from the interplay between multiple genes and gluten, the triggering environmental factor. HLA class II genes explain 40% of the heritable risk. Recently, we reported significant and replicable association to a common variant located in intron 28 of the myosin IXB (*MYO9B*) gene. Homozygosity for the at-risk allele confers a 2.3 higher risk to disease (P = 1.55 x 10^{-5}). MYO9B is an unconventional myosin that contains a Rho-GTPase activating domain which can negatively control Rho proteins. Rho proteins are involved in cytoskeletal modifications and tight junction assembly suggesting a role of MYO9B in the epithelial barrier. Hence, the genetic association may point to a primary impairment of the epithelial barrier, and may explain why immunogenic gluten peptides are able to pass through this barrier. A recent microarray study revealed enhanced neutrophil recruitment into the small intestine in both active and remission patients, further confirming our hypothesis of a genetic impairment of the intestinal epithelial barrier. Hence, ongoing genetic studies are currently focusing on tight-junction genes involved in epithelial barrier function. These studies are being complemented with gene expression studies using intestinal biopsies from coeliac patients and control individuals. Interestingly, *MYO9B* is also an attractive candidate gene for other inflammatory disorders since increased permeability of the epithelial barrier is also seen in e.g. multiple sclerosis, type 1 diabetes and asthma.

PL2.Circadian biology

***J. Meijer**;*
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The earth's rotation causes 24-hour cycles in many aspects of the physical environment, while the earth's revolution around the sun causes seasonal changes. Most living systems have developed circadian clocks to anticipate changes in the environment. Circadian rhythms can be observed in many tissues and organs, and are present at the level of gene expression, transmitter and hormone concentrations, enzyme activity, physiology and behavior. Light is the most important entraining signal from the environment and subserves entrainment to both daily and annual cycles. In mammals, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus. Individual neurons of the SCN are capable of generating circadian patterns on the basis of a molecular feedback loop in which 'clock genes' are inhibited by their protein products. The molecular oscillations result in circadian rhythms in membrane properties, resulting in rhythmic patterns in electrical impulse frequency of SCN neurons. Electrical impulse activity is an important output signal of the clock. We record electrical activity patterns in brain slices and in behaving animals implanted with microelectrodes, and investigated the ability of the SCN to code for -and synchronize to- daily and annual cycles. We obtained evidence that the SCN is composed of multiple single cell oscillators, and that the temporal distribution of these oscillators can be reconfigured by environmental changes. Within the SCN, sub-regions show different rates of entrainment to a shift in light-dark cycle, with the ventral SCN shifting more rapidly than the dorsal SCN. In addition, we show that areas outside the SCN can contribute critically to the resynchronization rate of the circadian system. We conclude that different properties of the circadian system arise at different levels of organization within the organism. While generation of circadian rhythms can be explained at the molecular level, synchronization to environmental signals requires neuronal networks.

PL3.Impact of genetic testing on breast cancer care, new developments

***H. Meijers-Heijboer**;*
Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands.

No abstract received.

PL4.Recent discoveries in the genetics of common diseases: selection and population history

***K. Stefansson**;*
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No abstract received.

PL5.Genetic Causes of Vascular Malformations

***M. Vikkula**;*
Human Molecular Genetics, Christian de Duve Institute & Université Catholique de Louvain, Brussels, Belgium.

Vascular malformations are *localized* errors of vascular development. They are often identified on the skin as "birthmarks" of various sizes and shapes. They usually slowly grow with the growth of the child. They may also be encountered in other organs, such as the liver, intestine and the brain. The lesions are consisted of tortuous vascular channels of various types, with continuous endothelium surrounded by various numbers of support cells. Most of these lesions occur sporadically, yet sometimes as part of a syndrome or as an inherited disorder. Genetic studies of such families have lead to the identification of a number of genes that can cause vascular malformations.

Our first discovery was the identification of the TIE2/TEK gene encoding an endothelial receptor tyrosine kinase to be responsible for hereditary mucocutaneous venous malformations ("cavernous hemangioma"). As a continuation to this work, we unraveled that a loss-of-function mutation in the VEGFR3 gene, encoding the vascular endothelial growth factor 3 receptor, is responsible for congenital hereditary lymphedema. We also linked mutations in the KRIT1 gene to cutaneous capillary-venous malformations associated with cerebral cavernous malformations. More recent work has lead to the identification of mutations in glomulin to be responsible for hereditary glomuvenous malformations ("glomangiomas"), SOX18 mutations to cause lymphedema-hypotrichosis-telangiectasia syndrome and RASA1 mutations to cause a newly recognized disorder, which associates atypical hereditary capillary malformations to arterio-venous anomalies (CM-AVM).

As the function of the genes identified using reverse genetics is often unknown, *in vivo* models are crucial for further dissection of the molecular pathways involved in these disorders. Such models will enable direct evaluation of the developmental function and significance of the genes in *vasculogenesis* and *angiogenesis*. In addition, murine models could serve for screening of novel therapeutic modalities.

PL6.Huntington's disease: molecular pathogenesis and therapeutic approaches

***G. Bates**;*
Medical and Molecular Medicine, School of Medicine, King's College London, London, United Kingdom.

Huntington's disease (HD) is an inherited, progressive neurological disorder caused by a CAG/polyglutamine repeat expansion for which there is no effective therapy. We have developed and characterised mouse models of HD that recapitulate many features of the human disease and have predicted novel aspects to human pathology. We are currently using these models to better understand molecular events in disease pathogenesis including somatic repeat instability and transcriptional dysregulation among others. Since the identification of the HD mutation, new targets for therapeutic intervention have been identified. We have established a battery of quantitative protocols for the preclinical assessment of compounds in the R6/2 mouse model of HD. Histone deacetylase inhibitors have emerged as potential HD therapeutics and are being assessed.

PL7.Systems biology approaches for the study of aging and age-related diseases

***R. Baumeister**;*
ZBSA – Freiburg Center for Systems Biology, University of Freiburg, Freiburg, Germany.

In the past years the various genome projects succeeded in delivering a rapidly increasing amount of gene information. However, the necessary functional analysis of this data with traditional research tools is far too slow to make optimal use of this information. In particular, it is required to understand the networks of gene regulations and protein interactions in order to interprete the phenotypic consequences of

e.g. mutants associated with disease. For this purpose, we have established a research platform using the soil nematode *C. elegans* to study the genome-wide consequences of genetic mutations. We exploit this model for the analysis of genetic components and signaling pathways affecting cellular and organismal aging and for the functional interpretation of mutants associated, in humans, with age-related degenerative disorders like Alzheimer's and Parkinson's Disease. *C. elegans* is particularly suited for the fast and genome-wide characterization of (disease-relevant) gene functions and networks, and it allows the testing of functional alterations in an otherwise intact organism. In addition, automation of particular experimental setups increases the throughput of data acquisition. The unique set of genetic, molecular, and genetic tools available for this organism, in combination with the high degree of sequence conservation between *C. elegans* and human genomes, aims at greatly enhancing our understanding of age-related disorders.

PL8.Humanity's Genes

***S. Brenner**;*
Brenner Laboratory, The Salk Institute for Biological Studies, La Jolla, CA, United States.
No abstract received.

PL9. Making eyes: lessons from ocular malformations

***V. van Heyningen**;*
MRC Human Genetics Unit, Edinburgh, United Kingdom.
Charles Darwin considered the eye an "organ of extreme perfection". The genes implicated in eye development are highly conserved across phyla. Study of ocular malformations led us to identify three major transcription factors which cause severe ocular malformations, in some cases with accompanying brain anomalies. PAX6 haploinsufficiency was shown to cause human aniridia; SOX2 and OTX2 were identified as significant causative genes for anophthalmia and microphthalmia. Detailed analysis of the mutations and observed phenotypes has highlighted key genetic mechanisms which we are pursuing using model organisms, predominantly mouse and zebrafish. Continuing exploration of patient mutations has also convinced us that humans provide excellent models for defining gene function and interactions. The search for additional candidate genes contributes significantly to the discovery of developmental pathways. Potential mechanisms for environmental modification of phenotype are also emerging in our studies. Our results illustrate the invaluable contribution made to our understanding of basic biology, as well as to clinical management, by the study of human disease, directly and in model organisms.

ESHG Concurrent Symposia

S01.Analysis of quantitative trait loci

***H. Göring**;*
Southwest Foundation for Biomedical Research, Department of Genetics and SNPRC, San Antonio, TX, United States.
No abstract received.

S02.Endophenotypes for cognitive ability

***E. de Geus**, D. Boomsma, S. van der Sluis, D. Smit, D. Posthuma;*
Dept. Biological Psychology & Centre for Neurogenomics and Cognitive Research, Vrije Universiteit, Amsterdam, The Netherlands.
Population variation in higher order cognitive ability, assessed by psychometric IQ, is largely determined by genetic variation. Recent whole genome searches have suggested that the high heritability of IQ derives from many genes with small effect. An endophenotype strategy may help counter the low power in linkage analyses implied by such a polygenic scenario. We aimed to develop endophenotypes for cognition in three domains of brain characteristics: bulk, speed and connectivity. Brain volumes, both total gray and white, showed the required correlation to IQ which also proved to be entirely genetic in origin. Strong sex differences in the relationship between IQ and brain volume, however, may complicate their use as endophenotypes. In the domain of procesing speed, a measure of early visual processing speed, visual inspection time, was correlated to performance IQ entirely through an underlying genetic factor. However, other indices of processing speed in the domain of EEG and ERP measures failed to genetically correlate to IQ, even when we found them to be highly heritable. With regard to corticocortical connectivity, linear (EEG coherence) and non-linear (synchronization likelihood) synchrony in brain activation has been tested so far. As with ERP latencies, substantial heritability was found, but these connectivity measures were only modestly correlated to IQ.
It is concluded that the endophenotype strategy is completely valid in theory but hard to put into practice.

S03.Gene Expression signatures identify clinically relevant subgroups of cancer - Examples from colon and bladder

***T. Orntoft**;*
Aarhus University Hospital, Aarhus, Denmark.
It has been a frustration in the clinical handling of cancer patients, that two highly similar looking cancers (same TNM stage, same histology) in two different individuals may show distinctly different disease courses. Two get a deeper look into the properties of cancers we have used a large prospectively collected tissue bank with clinical follow-up data, as well as modern microarray technology and bioinformatic data mining.
A number of gene expression signatures have been identified mainly in bladder and colon cancer using cross validation and a maximum likelihood method. For bladder cancer we have signatures for stage, progression, surrounding carcinoma in-situ, recurrence, treatment response etc. They have been identified on high density Affymetrix GeneChips and validated on independent test sets of patients using in-house gridded oligonucleotide microarrays. A multicenter European validation spanning from Spain to Sweden has been initiated and data from the first 400 patients will be reported, based on less than 500 genes in total.
As end points we have used either clinical upstaging based on TNM (tumor, node, metastasis) classification, or a clinical risk score that detects aggressive tumors early. In colon cancer we generated signatures for MSI/MSS, for heredity in MSI cases, as well as for predicting recurrence.
For some of the differentially expressed genes we have made in-vitro cellular studies of their biological function, and found several related to apoptosis or migration.
Individualized medicine is now a possibility as more and more drugs are being available for each cancer disease, and also individually planned follow up strategies are emerging. It requires, however, that we can differentiate between individual tumors, in terms of their predicted behaviour and treatment response. Gene expression signatures seem to be a promising tool for this purpose if used in a correct way.

S04.Ectodermal dysplasias

***M. Mikkola**;*
Developmental Biology Program, Institute of Biotechnology, Helsinki, Finland.
Ectodermal dysplasias (ED) represent a large group of rare genetic disorders with developmental abnormalities of the ectoderm and its derivatives such as teeth, hairs, nails, and sweat glands. Currently, in less than 30 of the approximately 200 EDs described to date the causative gene has been identified. Extensive recent progress has been made in understanding the biology of EDs associated with mutations in p63 and ectodysplasin (Eda) signalling pathway. Mutations in Eda cause the most common form of EDs, the X-linked anhidrotic ectodermal dysplasia, which is now known to involve deficient activation of the transcription factor NF-κB. Eda/NF-κB signalling is required for the early stages of ectodermal organogenesis - novel findings on this pathway will be discussed. Mutations in p63, a transcription factor similar to tumour suppressor p53, have been described in a number of autosomal dominant EDs including EEC (ectrodactyly-ED-clefting) and AEC (ankyloblepharon-ectodermal dysplasia-clefting) syndromes. p63 deficient mice die at birth and are featured by absence of stratified epidermis and lack of teeth, hairs, and mammary glands. Two fundamentally different types of p63 proteins are made: those containing an N-terminal transactivating (TA) domain similar to that found in p53 and those lacking this domain (ΔN). However, the relative contribution of the different p63 isoforms in epithelial organogenesis has remained an enigma. We have used mouse as a model to analyze the function of p63. Unexpectedly, we

found that the ΔN isoforms are expressed at high levels in embryonic ectoderm at all stages of the development of skin and its appendages, teeth and hairs. Comparative in situ hybridization approach revealed several genes that lie downstream of p63. The connection of p63 to other pathways implicated in EDs will be addressed.

S05.Ichthyosis

P. Steylen;
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No abstract received.

S06.Genetics of skin pigmentation and pigmentary diseases

R. Spritz;
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Because of the immediate visual impact, genetic disorders of pigmentation and the wide variation of normal pigmentation of humans, animals, and plants have long drawn the interest of geneticists, breeders, and the general public. Oculocutaneous albinism is perhaps the first genetic disorder ever described, and represents the first enzyme deficiency ever identified. Piebaldism is thought to be the first trait for which a pedigree was presented. The inheritance of red hair was one of the first normal human traits studied by early geneticists. Recent years have seen remarkable progress in this field. In the mouse, more than 130 genes are known to influence the pigmentary phenotype, and most of these have now been identified. In humans, five genes are now known for pure oculocutaneous albinism and twelve for oculocutaneous albinism associated with various systemic manifestations (Hermansky-Pudlak syndrome, Chediak-Higashi syndrome, or Griscelli syndrome). Seven genes are known to cause human congenital white-spotting disorders (piebaldism and various types of Waardenburg syndrome), and another four have been mapped but not yet identified. All of these human pigmentary diseases have direct parallels in the mouse, as well as in many other mammalian species. There has also been considerable recent progress in understanding the genetics of the most common human pigmentary disorder, vitiligo, an acquired white-spotting disorder of autoimmune origin that is highly associated with various other autoimmune/autoinflammatory diseases. Several genes have been epidemiologically associated with vitiligo, and at least two others have been identified by genetic linkage and positional cloning, perhaps opening up new avenues to treatment of this common disorder. Finally, evidence is mounting that some of these pigmentary disease genes may also play roles in normal variation of human pigmentation-of the hair, the skin, and the eyes, opening the door to study of these incredibly rich and complex human phenotypes.

S07.Mechanism and medical implications of RNA surveillance by Nonsense Mediated decay

A. Kulozik;
Universitätsklinik für Kinder- und. Jugendmedizin, Heidelberg, Germany.
No abstract received.

S08.RNA splicing in cancer

J. P. Venables;
Institution of Human Genetics, Newcastle-upon-Tyne, United Kingdom.
More than 90% of protein coding transcripts undergo removal of introns and splicing together of exons. This process depends on recognition of multiple signals, especially within 200 nucleotides of exon-intron boundaries, and mutation of these signals is thought to account for around 15% of genetic disease. 'Alternative splicing' is the process whereby the majority of genes encode more than one polypeptide. Despite being from the same gene these variant proteins can be functionally antagonistic and choreographed changes in isoform balance can lead to specific modes of differentiation. In several tumour types the ratio of splice variants, in genes involved in proliferation, apoptosis or motility, is altered and has independent prognostic value. Moreover, inducing splice form imbalances characteristic of tumours can lead to malignant behaviour in cultured cells. Therefore unbalanced alternative splicing of multiple target pre-mRNAs caused by dysregulation of splicing factors could be the primary cause of cancer in some instances.

S09.Genetics of variation in human gene expression

V. G. Cheung;
Pediatrics, University of Pennsylvania, The Children's Hospital of Philadelphia, Department of Pediatrics, Philadelphia, PA, United States.
No abstract received.

S10.Taste Genetics: Insights in Individual Taste Worlds with Implications for Diet and Health

B. Tepper;
Department of Food Science, Rutgers University, New Brunswick, NJ, United States.
Taste is critical for the identification and selection of food and is therefore essential for human survival. Humans have an innate preference for sweet taste, a source of calories, and an innate aversion to bitter taste, which might protect against the consumption of toxic substances. Aside from these innate responses, there are large individual differences in taste preferences that are thought to be genetically determined. The ability to taste bitter thiourea compounds and related chemicals is a well-known human trait. The majority of individuals perceive these compounds, typified by the bitterness of 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC), as moderately-to-extremely bitter. Approximately 30% of the population is taste blind to these substances. PROP/PTC tasters are more sensitive to a wide range of oral sensations including other bitter tastes, sweetness, spiciness of chili peppers, astringency of alcohol and the texture of fats. Tasters typically show lower preferences for foods with these taste qualities than non-tasters who show the opposite set of responses (lower taste sensitivities and higher preferences for these sensory qualities). We have used PROP tasting as a screening tool to understand genetic variation in food preferences that may have long-term implications for diet and health. We have shown, for example, that the non-taster phenotype is associated with higher fat and energy intakes in children and higher body weights in adults, especially women. These findings suggest that the PROP/PTC bitter taste phenotype may be a marker for increased weight gain and obesity.

S11.Genetics of smell

D. Lancet;
Crown Human Genome Center, Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel.
No abstract received.

S12.Genetics of pain perception

A. Dahan;
Leiden University Medical Center, Leiden, The Netherlands.
No abstract received.

S13.Genetic control of infectious disease in humans

A. Hill;
Wellcome Trust Centre for Human Genetics, Oxford U, Oxford, United Kingdom.
No abstract received.

S14.Mice, microbes and models of infection

R. Balling;
Institute für Säugetiergenetik, GSF, Neuherberg, Germany.
No abstract received.

S15.From idiopathic infectious diseases to novel primary immunodeficiencies

J. L. Casanova[1,2];
[1]Laboratoire de Génétique Humaine des Maladies Infectieuses, Université de Paris René Descartes-INSERM U550, Faculté de Médecine Necker, Paris, France, [2]Unité d'Immunologie et d'Hématologie Pédiatriques, Hôpital Necker Enfants Malades, Paris, France.
Primary immunodeficiencies are typically seen as rare monogenic conditions associated with detectable immunological abnormalities, resulting in a broad susceptibility to multiple and recurrent infections caused by weakly pathogenic and more virulent microorganisms. By opposition to these conventional primary immunodeficiencies, we describe non-conventional primary immunodeficiencies as Mendelian conditions manifesting in otherwise healthy patients as a narrow

susceptibility to infections, recurrent or otherwise, caused by weakly pathogenic or by more virulent microbes. Conventional primary immunodeficiencies are suspected on the basis of a rare, striking, clinical phenotype and are defined on the basis of an overt immunological phenotype, often leading to identification of the disease-causing gene. Non-conventional primary immunodeficiencies are defined on the basis of a more common, less marked clinical phenotype, which remains isolated until molecular cloning of the causal gene reveals a hitherto undetected immunological phenotype. Similar concepts may be applied to primary immunodeficiencies presenting other clinical features, such as allergy and auto-immunity. Non-conventional primary immunodeficiencies thus expand the clinical boundaries of this group of inherited disorders considerably, suggesting that Mendelian primary immunodeficiencies are more common in the general population than previously thought, and may affect children with a single infectious, allergic, or autoimmune disease.

S16.Constitutional aneuploidy and cancer predisposition

N. Rahman;
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No abstract received.

S17.Spindle checkpoint proteins and their multiple roles in regulating chromosome segregation

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Accurate segregation of chromosomes during cell division is essential to maintain genomic stability. Cells have developed a surveillance mechanism that monitors attachment of chromosomes to the spindle and delays anaphase onset until chromosomes congress properly. The basic signalling components of the Spindle Assembly Checkpoint (SAC) have been shown to be and include the Bub and Mad family of proteins. When chromosomes are not correctly attached, these proteins o provide an inhibitory signal that prevents activation of the ubiquitin dependent ligase complex (APC/C) inhibiting mitosis exit. We have set out to dissect the SAC in Drosophila through functional studies of Bub3, BubR1 and Mad2. The results indicate that apart from their function in the SAC, these proteins appear to have other roles during mitosis.

In vivo studies show that Mad2 is required during mitosis for the correct timing of prometaphase independently of kinetochore localization. Bub3 was found to be involved in entry and progression through mitosis by preventing premature degradation of cyclins and BubR1 appears to regulate the stability of kinetochore-microtubule interaction during prometaphase. Furthermore, genetic analysis of BubR1 has revealed that the SAC it self is required during early embryo development, as well as for the arrest of the polar body after meiosis. We also find that BubR1 is essential during early stages of female meiosis to prevent chromosome missegregation. Overall our results suggest that SAC proteins are used in different cellular and developmental contexts to maintain genomic stability.

S18.Regulating mitosis by proteolysis

J. Pines;
Wellcome Trust/Cancer Research UK, Cambridge, United Kingdom.

No abstract received.

S19.Cascade screening: whose information is it anyway?

G. de Wert;
Maastricht University, Maastricht, The Netherlands.

No abstract received.

S20.Autonomy and prenatal testing decisions

E. Dormandy, *T. Marteau*;
King's College London, London, United Kingdom.

The importance of acting in line with individual values is central to the principle of autonomy, a highly valued, individualistic concept, governing encounters in medicine in developed countries. The congruence between values and action is also central to the concept of informed choice. Increasingly the patient population in developed countries encompasses people from a variety of cultures including those in which group perspectives can be valued more highly than those of the individual. Embedding value-behaviour consistency at the heart of informed choice could therefore be inappropriate when evaluating decision making across cultural groups.

Studies in the US, Europe and Australia show that uptake of screening for fetal abnormalities is lower amongst women from minority ethnic groups. It is commonly assumed that this lower uptake reflects more negative attitudes towards testing in these women. Using a validated measure of attitudes towards testing we found no difference in attitudes towards undergoing the test but rather a lower consistency between values and action in women from South Asia compared with white women. One interpretation of these findings is that women from minority ethnic groups are less likely to act consistently with their values. Another explanation is that the measure of values did not capture the values importance to women from South Asia, a culture characterised more by collectivism than individualism. If this is the case it raises a question of whether acting in line with collectivist values can or indeed should be incorporated into the more individualistically based concept of informed choice.

S21.Shared decision making in clinical genetics.

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Wessex Clinical Genetic Service, Southampton, United Kingdom.

One of the main aims of a clinical genetic service is to offer patients informed choices. Historically, a delivery mode of 'non-directive counselling' has been advocated in part to distance the speciality from any association with the eugenic movement but also because there was little evidence basis for effectiveness of particular interventions. As such an evidence basis accumulates and as more and more complex diagnoses and predictions can be made, counselling through 'shared decision making (SDM)' has been advocated. SDM is thought to be particularly appropriate in situations of uncertainty where several reasonable clinical alternatives exist; the patient and clinician explore these and come to a shared decision on a course of action which incorporates the patient's life experiences and intuitions. This process thus enhances autonomy by informing the consent process. However, there are several characteristics of clinical genetics that may make shared decision more difficult to achieve than in other areas of medicine. Guilt, worry about stigma or discrimination may make decisions in genetics more difficult and the underlying concepts and mechanisms may be more difficult to understand than non-genetic tests. The shared nature of genetic test results among family members may mean that one result points to others who might benefit from interventions. Certain interventions (such as thyroidectomy in MEN2 carriers) may be so much more beneficial than any other alternative, that the decision making model moves to a professional recommendation. Such challenges to autonomy and decision making will be explored.

S22.Age-related Macula degeneration

C. Klaver;
Erasmus MC, Polikliniek Oogheelkunde, Rotterdam, The Netherlands.

No abstract received.

S23.Parkinson disease and LRRK2

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Genetic findings in rare inherited forms of Parkinson's disease (PD) have greatly contributed to our understanding of the molecular pathogenesis of this disease.

We have recently identified mutations in the gene for LRRK2 (leucine-rich repeat kinase 2) as the so far most common cause of dominantly inherited PD. Mutations are located in highly conserved domains of the gene. The LRRK2 protein belongs to the ROCO protein family, and includes a ras domaine (ras of complex proteins) and a protein kinase domain of the MAPKKK class and several other major functional domains. Extensive sequencing of families and sporadic patients by many groups now shows that LRRK2 mutations account for about 10 to 15% of dominant PD, and for 1 to 2% of typical sporadic late-onset PD. Pathologically, most patients show brainstem dopaminergic

degeneration accompanied by typical Lewy body pathology, but occasionally the picture is that of a diffuse Lewy body disease, nigral degeneration without distinctive histopathology and even progressive supranuclear palsy.
Recent analyses showed that LRRK2 is an active kinase, and that pathogenic mutations may actually increase kinase activity, potentially opening up novel approaches to therapy.

S24.*APP* locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy

A. Rovelet-Lecrux[1], D. Hannequin[1,2], G. Raux[1], N. Le Meur[3], A. Laquerrière[4], A. Vital[5], C. Dumanchin[1], S. Feuillette[1], A. Brice[6], M. Verceletto[7], F. Dubas[8], D. Campion[1,9], ***T. Frebourg***[1];
[1]Inserm U614-IFRMP, Faculty of Medicine, Rouen, France, [2]Department of Neurology, University Hospital, Rouen, France, [3]Laboratory of Cytogenetics, EFS Normandy, Bois-Guillaume, France, [4]Department of Pathology, University Hospital, Rouen, France, [5]Department of Pathology, University Hospital, Bordeaux, France, [6]Inserm U679, Salpétrière Hospital, Paris, France, [7]Department of Neurology, University Hospital, Nantes, France, [8]Department of Neurology, University Hospital, Angers, France, [9]Department of Research, CHSR, Sotteville les Rouen, France.

The development of Alzheimer disease (AD) in patients with trisomy 21 led us to hypothesise that the *APP* locus located on chromosome 21q21 might be affected by gene dosage alterations in a subset of demented patients. We therefore analysed the *APP* gene using QMPSF (Quantitative Multiplex PCR of Short Fluorescent Fragments) in 12 unrelated autosomal dominant early-onset Alzheimer disease (ADEOAD) cases without *PSEN1*, *PSEN2* or *APP* mutation, 70 unrelated familial late onset AD cases and 100 healthy control subjects. We found a duplication of the *APP* locus in 5 families with ADEOAD and cerebral amyloid angiopathy (CAA). Among families, the duplicated segments had a minimal size ranging from 0.58 to 6.37 Mb and contained from 5 to 12 annotated genes. Dementia of AD type (mean age of onset 52 ± 4.4 years) was associated, in some cases, with lobar intracerebral haemorrhages. Remarkably, retrospective examination of the patients harbouring the chromosome 21 segmental duplication did not reveal any clinical feature suggestive of Down syndrome. Brains from patients with *APP* duplication presented abundant parenchymal and vascular deposits of Aβ peptides. These data demonstrate that *APP* gene dosage alteration *APP* is sufficient to induce Aβ deposits and ADEOAD associated with CAA. This validates the amyloid cascade model according to which AD results from an abnormal production and/or aggregation of the Aβ peptide. After the report of alpha synuclein triplication in Parkinson disease, this is a further evidence that gene dosage alterations are involved in the genetic determinism of neurodegenerative disorders caused by protein or peptide accumulation.

S25.Segmental Duplication and Human Genome Variation

E. E. Eichler;
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The abundance of segmental duplications within the human genome (~4%) with relatively high sequence identity (>95%) suggests that duplicative transposition or gene conversion of large segments of DNA must have been a relatively common form of genetic variation during hominoid evolution. Moreover, the presence of such large blocks of sequence at multiple regions creates the opportunity for non-allelic homologous recombination events that may have significantly restructured the genome in ancestral and present-day populations. I will present a survey of structural variation within the human population within these regions of the genome based on two different approaches. Using BAC-based array comparative genomic hybridization, I will present a survey of copy number variation of these regions within the human population and show that such regions are particularly enriched for large-scale variation. The analysis identifies ~40/130 regions that appear to be "recalcitrant" to copy number changes among normals-21 of which are discovered to be variant among probands with idiopathic mental retardation. We suggest that some of these represent novel genomic disorders. Using paired-end sequence analysis, I will provide a first generation view of finer-scale structural variation within the human genome (inversions, deletions and insertions > 10 kb in length) based on a detailed analysis of two human-genomes by paired-end clone sequence analysis. Our data suggests that this form of variation is common within and between primate species and preferentially occur within duplicated and often gene-rich regions of the genome. The nature and pattern of such variation will likely be an important consideration in genetic association studies of human disease.

S26.Deconstructing deletion syndromes: new techniques and old karyotypes

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Dept. Human Genetics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

New molecular cytogenetic techniques like arrayCGH and MLPA were primarily introduced to search for submicroscopic chromosome aberrations in patients with unexplained mental retardation. However these techniques may also improve phenotype-genotype studies in microscopically visible chromosome aberrations. The exact determination of breakpoints needed for these studies used to be very time-consuming and only feasible for rather common cytogenetic syndromes. Size and localisation of the chromosomal aneuploidies can nowadays be determined with very high accuracy by tiling path arrayCGH in one single test run.
With the availability of very high-resolution genotyping, phenotyping with an equally high accuracy is needed to fully benefit from the advantages of these new techniques. The combination of high-resolution genotyping with detailed phenotyping will enable us to localise genes for specific traits in cytogenetic syndromes. Examples are the determination of the critical region for congenital aural atresia with a size of 2.3 Mb on chromosome 18q22.3 and the critical region for trigonocephaly on 9p22.3. High quality and standardized collection of molecular cytogenetic and detailed clinical information will also enable aneuploidy studies in less common chromosomal aberrations. Van Buggenhout nicely demonstrated that accurate phenotyping and genotyping in as less as four patients can already reveal a small region of interest for a behavioural phenotype (2q32.3).
A short overview of classical phenotype-genotype studies in chromosomal syndromes will be presented followed by a review of studies using new techniques. Advantages and limitations of the new approach and the need for sophisticated phenotyping and data collection will be discussed.

S27.Segmental Duplications in 22q11 Mediate Deletions, Translocations and Genomic Instability

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No abstract received.

S28.Probing the genetic basis of human brain evolution

B. Lahn;
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No abstract received.

S28.Vertebrate chromosome evolution since our last common ancestor

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All vertebrates derive from a common ancestor that lived about 450 million years ago. The genome of this ancestor was modified by chromosome rearrangements in different lineages, and the exquisite details provided by the availability of the genome sequence for many modern vertebrate species makes it possible to deduce most of these chromosome rearrangements. For example, the sequence of the pufferfish Tetraodon nigroviridis genome showed with great clarity that a whole genome duplication took place in an early fish ancestor that possessed only 12 chromosomes. The two signatures of this event materialize when examining the distribution of duplicated genes on the 21 Tetraodon chromosomes on the one hand, and when comparing the distribution of human and Tetraodon genes on the other hand.
A systematic comparison of gene organisations between fish and other vertebrates thus provides a powerful basis to reconstruct their ancestral

genome and trace its evolution in the fish, bird or mammalian lineages. The general scenario follows some striking patterns, such as slow rate of rearrangements along the fish and bird lineages, and a faster rate along the mammalian line and provides a reference framework to follow the distribution of genes and gene families in different lineages.

S30.The complexity of human genes

***R. Guigo**, and the ENCODE (ENCyclopedia Of Dna Elements Project Consortium) Genes & Transcripts Analysis Group;*
Centre de Regulació Genòmica, Institut Municipal d'Investigació Mèdica-Universitat Pompeu Fabra, Barcelona, Spain.

Transcribed regions have been long been regarded as a distinguishing characteristic of functional portions of the human genome. Increasing experimental evidence, however, indicates that larger portions of the genome are being transcribed than originally believed. As part of the Encyclopedia of DNA Elements (ENCODE) project, the sites of transcription in the non-repeat sequences across a representative 1% of the human genome has been determined in a large number of different cell line/tissue samples using of high throughput transcription interrogation technique. In addition, a detailed annotation of the protein coding content of the ENCODE regions has been obtained through a combination of computational, experimental and manual methods. Overall, at least 90% of the ENCODE regions appear to transcribed as primary nuclear transcripts, and about 15% are present as mature processed polyadenylated transcripts. Interestingly up to 30% of these sites of transcription have not been previously identified. Explorations of the 5' annotated boundaries of the protein coding genes completely contained within the ENCODE regions revealed that a large fraction of them initiate their transcription in a tissue-specific manner at unannotated distant sites: 30% of these distal alternative exons extend more than 100,000 bp away, often overlapping regulatory regions of other upstream genes. Overall, through these studies, a complex organization of lattice-like networks of transcription across the genome is revealed.

S31.Separating means from ends: turning back to the goals of genetic counseling

***S. Shiloh**;*
National Institutes of Health, National Human Genome Research Institute, Social & Behavioral Research Branch, Bethesda, MD, United States.

The quality of genetic counseling must be related to the definition of its goals. A modern definition of genetic counseling sees it as a psycho-educational process centered on genetic information, in which the goal is to facilitate clients' ability to use genetic counseling in a personally meaningful way that minimizes psychological distress and increases personal control. Consequently, psycho-educational process measures and commonly studied amount and accuracy of the information gained in genetic counseling should be regarded as predictor variables of the ultimate outcomes defined by measures of personal control, coping and well being. Separating information from decision as outcomes of counseling is artificial, since information is provided in genetic counseling not as an end in itself, but as a means of facilitating a personally satisfactory decision. Research findings will be presented on the helpfulness of the information received in counseling for facilitating counselees' decisions, and on the level of perceived personal control following genetic counseling and its effects on subsequent coping.

S32.Listening to consumers in genetic healthcare - an audit tool to support measurement of outcomes

***H. Skirton**;*
Faculty of Health and Social Work, Taunton, United Kingdom.

Evaluation of the outcomes of healthcare is an important component of both service audit and clinical research. Genetic services do not conform to a traditional health service model and assessment may be more challenging than it is for many other services because of the lack of readily-measurable outcomes. An effective way of assessing consumer needs and evaluating services from the client's perspective is required. Changes in reproductive behaviour, knowledge of recurrence risk figures and satisfaction with the consultation have all been used as outcome measures in the past. However, at least some of these constructs may be inappropriate because they contradict the philosophy of client choice that underpins genetic healthcare. Previous studies point to a lack of baseline knowledge of genetics in the general population and confirm the need for a strong informational component to the service. However, qualitative research has indicated that important outcomes from the client's perspective also relate to changes in psychological adaptation to the genetic condition or genetic risk. These studies were the foundation for the development of the Genetic Healthcare Outcomes Questionnaire. Six main factors contributing to the outcome of the service from the client's perspective. These were labelled i) enhanced understanding ii) positive psychological change iii) respect for autonomy iv) adaptation v) disequilibrium and vi) value of contact. These outcomes are relevant to a range of healthcare services and could be used to counter the argument for genetic exceptionalism.

S33.What works, and why, in Genetic Counselling? The need for theory

***S. Michie**;*
Department of Psychology, and Institute for Human Genetics and Health, University College London, London, United Kingdom.

To ***judge*** the quality of genetic counselling, we need to know its objectives, and be able to measure them. Arguably, establishing quality requires measuring process as well as outcome. To ***improve*** the quality of genetic counselling, we definitely need to measure process. Thus, developing quality requires the ability to answer "What do we want to achieve?" (outcomes), "How do we know when we've achieved it?" (measures) and "How did we achieve it?" (process)

Decisions about what outcomes to measure can be made on pragmatic grounds e.g. what patients, health professionals and/or policy makers consider to be desirable outcomes. Decisions about what processes to measure require knowledge of relevant theories e.g. of communication, risk perception and decision making. Without this, attempts to improve genetic counselling will be a rather "hit and miss" affair, and there will be limited possibilities of integrating and accumulating our knowledge. Developing a theoretical understanding of the processes of genetic counseling will help us build a picture of "What works for whom under what circumstances?"

The advantages of using an explicit theoretical framework will be illustrated by an atheoretical evaluation of the process and outcome of genetic counseling, and a theoretically based study of informed choice.

S34.Targeting the DNA repair defects in tumours

***A. Ashworth**;*
The 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. These approaches may also be applicable to sporadic cancers harbouring DNA repair defects. Progress towards developing these 'synthetic lethal' approaches will be discussed.

S35.Exploring the role Wnt/β-catenin signaling in intestinal and mammary cancer stemness

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Experimental Animal Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.

Breast and colon cancers are generally thought to arise from normal epithelial tissues through a stepwise accumulation of genetic alterations in oncogenes and tumor suppressor genes. However, this model does not take into account other essential characteristics of human cancers, namely their vast intratumor cellular heterogeneity and the role played by a minority of cells, the cancer stem cells, in determining local invasion into surrounding tissues and distant organ sites. The Wnt/β-catenin signal transduction pathway is known to play a central role in self-renewal and differentiation during embryonic development, and in the maintenance of many stem cell niches in adulthood. Accordingly, mutations in several Wnt-genes are rate-limiting to trigger tumorigenesis in the corresponding tissues.

To study how different dosages of Wnt signaling activation may influence multi-organ tumorigenesis, we have generated several hypomorphic alleles of the *Apc* tumor suppressor gene by gene targeting. Notably, while in general *Apc* mutations result in intestinal cancer both in man and mouse, the novel *Apc*^{1572T} mutation, resulting in a 175 kDa truncated protein lacking the axin-binding motifs but still encompassing 3 β-catenin down-regulating domains, does not lead to predisposition to intestinal tumors. However, the majority of *Apc*$^{+/1572T}$ females spontaneously develop multi-focal and rapidly growing mammary tumors. Notably, *Apc*^{1572T} mammary tumors also form lung metatstases. Both the primary tumors and their metastases were found to encompass myoepithelial, squamous, and luminal epithelial types, and were classified as lobular carcinomas with different degrees of metaplastic squamous differentiation. As previously observed for intestinal cancers, intracellular β-catenin accumulation, the earmark of canonical Wnt signaling activation, was found to be heterogeneous within the tumor mass. However, when lung micro-metastases (20-100 cells in size) were analyzed by IHC and compared with the macrometastases and with the primary mammary lesions, differentiation was found to be significantly reduced whereas almost every cell showed cytoplasmatic and/or nuclear β-catenin accumulation. These results indicate that the specific Wnt signaling dosage encoded by the *Apc*^{1572T} mutation differentially affects homeostasis of the mammary stem cell compartment and triggers tumor initiation, progression, and metastasis possibly by activating cancer stem cells. These novel data will be discussed also in view of recent results from our and Dr. S. Robine's (Paris, France) laboratories indicating a similar role for Wnt/β-catenin signaling in establishing cancer stem cells in an *Apc*^{1638N}/*KRAS*^{V12G} mouse model for intestinal tumorigenesis and liver metastases (KP Janssen, et al., *submitted*).

S36.Interference with signal transduction events emanating from the ErbB2 receptor sensitizes breast cancer cells to taxol induced cell death

***B. Groner**, C. Borghouts, C. Kunz;*

Georg Speyer Haus, Institute for Biomedical Research, Frankfurt, Germany.

The ErbB2 receptor tyrosine kinase is overexpressed in approximately 30 percent of breast tumor cases and its overexpression correlates with an unfavorable prognosis. A major contributor to this course of the disease is the insensitivity of these tumors towards chemotherapy. Monoclonal antibodies, inhibiting the ligand induced activation of the receptor and tyrosine kinase inhibitors acting on the intrinsic enzymatic activity of the intracellular domain have been developed as targeted drugs. Both have been shown to be beneficial for breast cancer patients. We targeted a third aspect of receptor function; its association with intracellular signaling components. For this purpose we selected peptide aptamers which specifically interact with defined domains of the intracellular part of the receptor. The peptide aptamers were selected from a random peptide library using a yeast two-hybrid system with the intracellular tyrosine kinase domain of ErbB2 as a bait construct. We investigated the functional consequences of the aptamer interaction with the ErbB2 receptor within tumor cells. The aptamer sequences were either expressed intracellularly or introduced into the cells as recombinant aptamer proteins. The aptamers co-localized with the intracellular domain of ErbB2 within cells. They reduced the extent of AKT kinase induction upon heregulin treatment of the cells. AKT is a signaling component downstream of the heregulin induced ErbB2-ErbB3 heterodimer in MCF-7 breast cancer cells. High AKT activity is responsible for the enhanced resistance of ErbB2 overexpressing cancer cells towards chemotherapeutic agents. Peptide aptamer interference with AKT activation resulted in the restoration of regular sensitivity of breast cancer cells to taxol.

S37.On genes speech and language

***S. Fisher**;*

Wellcome Trust Centre for Human Genetics, Oxford University, Oxford, United Kingdom.

Genes that are implicated in speech and language impairment can provide molecular windows into neural mechanisms contributing to human communication. We have shown that people who have FOXP2 missense or nonsense mutations develop major problems with controlling the complicated mouth movements needed for speech, along with deficits in many aspects of language and grammar. The gene encodes a conserved transcription factor that appears to help regulate development of a subset of neuronal circuits in a wide range of non-speaking vertebrates, but evidence suggests that its role may have been modified during human evolution. It is emphasised that FOXP2 is not the mythical "gene for language", but instead represents one piece of a complex evolutionary puzzle, involving multiple factors. My laboratory are using FOXP2 as an entry point for investigating neuronal pathways underlying speech and language acquisition, by adopting a range of complementary functional genetic techniques, from cell-lines to mutant mice. The FOXP2 story shows that understanding of language origins requires a multidisciplinary perspective, integrating psychology, neuroscience, genetics, developmental biology and evolutionary anthropology.

S38.Molecular, cellular, and circuit mechanisms underlying the storage of remote memories in cortical networks

***A. J. Silva**;*

Departments of Neurobiology, Psychiatry and Psychology, Brain Research Institute, University of California, Los Angeles, CA, United States.

While the molecular, cellular and systems mechanisms required for the initial processing of memory have been intensively investigated, those underlying remote storage remain elusive. I will present neuroanatomical, pharmacological and genetic results demonstrating that specific areas of the cortex play a critical role in remote memory. Imaging of activity dependent genes shows that specific areas of the cortex are activated by remote memory and that this activation is impaired by a null alpha-CaMKII mutation that blocks remote memory. Accordingly, reversible inactivation of specific cortical structures in normal mice disrupts remote memory without affecting recent memory. Additionally, a genetic screen revealed several mutations that affect specifically remote memory without disrupting acquisition or initial hippocampal-dependent consolidation.

S39.Dyslexia

***G. Schulte-Körne**;*

Department of Child and Adolescent Psychiatry and Psychotherapy, Philipps University, Marburg, Germany.

Dyslexia is a specific disorder in learning to read and spell which is not the direct result of other disorders such as mental retardation or lesser impairments in general intelligence, gross neurological deficits, uncorrected visual or auditory problems, or emotional disturbances or inadequate schooling.

A genetic involvement in dyslexia has long been evident from studies showing familial clustering of the disorder and more recently through twin studies. Linkage studies have identified several chromosomal regions of interest. Genetic loci have been mapped to at least nine different chromosomes (DYX1-DYX9) by several independent studies. Candidate genes have so far been proposed for DYX1(DYX1C1, ROBO1) and DYX2 (VMP, DCDC2, KIAA0319, TTRAP, THEM2).

One of the major difficulties in dissecting the genetic aetiology of learning disorders is that the clinical definitions comprise a number of heterogeneous conditions and phenocopies. In order to investigate genetically informative endophenotypes in a German multicenter study a broad spectrum of dyslexia related phenotypes including phonological decoding, phoneme awareness, orthographic processing, short-term memory, rapid naming and mathematical abilities were investigated in large sample of 287 German dyslexia families. The interrelationship

between the component phenotypes using correlation and principal component analyses (PCA), estimated familiality for phenotypes as well as for the factors suggested by PCA were investigated.
In the future, a multidisciplinary project (www.neurodys.com) will investigate the biological basis of dyslexia by mapping dyslexia susceptibility genes, identifying risk-conferring genes used to understand gene-gene and gene-environment interactions and gene-specific contributions to a variety of neurobiological correlates of dyslexia. Further, the environmental risk factors will be investigated and the neuroscientific basis providing the prerequisites of reading and spelling development and the central stages of becoming a fluent reader will be studied. These combined efforts will improve the basis for the development of successful diagnostics and therapies.

S40.New insights into molecular genetics of ARVC

***B. Gerull**;*
Max-Delbrueck Center for Molecular Medicine, Berlin-Buch, Germany.
Patients with arrhythmogenic right ventricular cardiomyopathy (ARVC) often present with unexplained ventricular arrhythmias, syncope or sudden cardiac death, the most feared clinical manifestation of disease. ARVC is pathologically characterized by progressive replacement of cardiac myocytes with fibrofatty tissue and occurs in rare syndromic and, more commonly, non-syndromic forms. Syndromic forms of ARVC (e.g. Naxos disease and others) are caused by mutations in plakoglobin or desmoplakin. Non-syndromic forms are mostly transmitted as an autosomal dominant trait. Three rare disease genes encoding the cardiac ryanodine receptor, TGFß3 and desmoplakin can cause autosomal dominant transmitted ARVC. Additional disease loci have been suggested containing still unknown ARVC disease genes. Recently we have identified a very common disease gene for ARVC, plakophilin-2 (*PKP2*).
Based on observations that Pkp2 deficient mice died early between E10-11 due to reduced trabeculation, disarrayed cytoskeleton, ruptured cardiac walls with blood leakage into the pericardial cavity, we screened ARVC patients for defects in *PKP2*. In 32 of 120 ARVC (26%) patients insertion, deletion, nonsense, missense, and splice site mutations were identified in the human *PKP2* gene. Western blot analyses in a case of a deletion *PKP2* mutation revealed reduced amounts of PKP2 suggesting haploinsufficiency as genetic mechanism. The observation of severely affected adolescents (including young individuals suffering from sudden cardiac death) and mutation carriers who remained healthy until old age points to a highly variable disease penetrance in the presence of *PKP2* mutations.

S41.The role of Tbx1 in DiGeorge syndrome

***A. Baldini**, Z. Zhang, L. Chen, F. Vitelli, E. A. Lindsay;*
Institute for Biosciences and Technologies, Texas A&M University Health Sciences Center, Houston, TX, United States.
The 22q11.2 Deletion / DiGeorge syndrome (DGS) is a relatively common “genomic” disorder that results from heterozygous deletion of a 3 Mbp segment of chromosome 22. Of the more than 30 genes deleted in this syndrome, *TBX1* is the only one that has been found to be mutated in some patients with a DGS-like phenotype, suggesting that *TBX1* haploinsufficiency is a major contributor to the syndrome’s phenotype. The main cardiovascular abnormalities in DGS and *Tbx1* mouse mutants concern the aortic arch and the cardiac outflow tract (OFT). Using a series of engineered alleles of *Tbx1* in the mouse, we have gathered data suggesting that the pathogenetic mechanisms underlying the two types of defects are different. Aortic arch and great arteries patterning is more sensitive to Tbx1 dosage than OFT morphogenesis, and requires *Tbx1* in pharyngeal epithelia early in embryonic development. OFT morphogenesis requires *Tbx1* function in the pharyngeal mesoderm and at a slightly later developmental stage. Tbx1 is required, cell non-autonomously, for the formation of the pharyngeal arch arteries that later remodel into the aortic arch. An *Fgf8-Tbx1* interaction is critical for the remodeling of these arteries. Concerning OFT development, our data demonstrated that *Tbx1* is required to sustain proliferation of cardiomyocyte precursors destined to the OFT. Loss of function of the gene in those cells resulted in severe morphogenetic defects of the OFT. In addition, cell fate mapping experiments showed that *Tbx1* mutation also affects cell lineage distribution within the right ventricle, a potential mechanism for the observed malalignment of the ventricles with the OFT.

S42.Concepts of cardiac development

***A. Moorman**;*
Department of Anatomy & Embryology, Academic Medical Center, Amsterdam, The Netherlands.
In this presentation we discuss the formation of the synchronously contracting chambered heart from a peristaltically contracting linear heart tube. It is proposed that members of the T-box family of transcription factors play a crucial role in the formation of the building plan of the formed heart. Tbx5 may confer veno-arterial polarity to the heart tube, whereas Tbx2 initially and Tbx3 in later developmental stages prevent the cardiac inflow tract, atrioventricular region, outflow tract, as well as the cardiac inner curvatures from chamber differentiation. With the exception of the outflow tract that becomes incorporated into the ventricles, these regions contribute to the cardiac conduction system. Tbx1 specifically is involved in the formation of the outflow tract, whereas Tbx18 is involved in the formation of the systemic venous tributaries.
One of the most fascinating aspects in the formation of the heart is the very early development of the electrical patterning as can be registered by the ECG, which is the registration of the rhythmic waves of depolarising activity over the cardiac muscle. In the mature heart the conduction system is held responsible for the rhythmic excitations and contractions. However, in chicken embryos a sinusoidal type of ECG can already be derived from the linear heart tube stages at about two days of development onward, and less than one day later when chamber formation has just been initiated, an adult type of ECG can be monitored.
The question can thus justifiably be asked whether the presence of an adult type of ECG in these early embryonic hearts implies the presence of a conduction system. Generally, the conduction system is defined as the system of specialized myocardial tissues responsible for initiation and propagation of the sinus impulse. If we accept this functional definition, then the early embryonic heart has a conduction system, which is already in place, because there is an adult type of ECG. On the other hand, if we would apply strict anatomical and histological definitions, the embryonic heart undeniably lacks a conduction system. This field of tension concerning the recognition of the conduction system using physiological versus anatomical / histological criteria has led to many controversies in the field of the development of the ‘cardiac specialized tissues’, which largely relate to definitions and semantics. In this contribution we discuss the specification of the embryonic areas that will become chamber and those areas that will not form chambers and participate in the formation of the distinct components of the conduction system.

ESHG Concurrent Sessions

C01. Genomewide Quantitative Trait Association Study of Cardiac Repolarization (QT-interval) Identifies and fine maps a QTL to the CAPON/NOS1AP Gene

***A. S. Pfeufer**[1], D. E. Arking[2], W. Post[2], W. H. L. Kao[2], M. Ikeda[2], K. West[2], C. Kashuk[2], M. Akyol[3], S. Perz[3], S. Jalilzadeh[1], T. Illig[3], C. Gieger[3], H. E. Wichmann[3], E. Marban[2], P. M. Spooner[2], S. Kaab[4], A. Chakravarti[2], T. Meitinger[1];*
[1]TU Munich, Munich, Germany, [2]Johns Hopkins Medical Institutes, Baltimore, MD, United States, [3]GSF National Research Center, Neuherberg, Germany, [4]LMU Munich, Klinikum Grosshadern, Munich, Germany.
Aim: To map QTLs for cardiac repolarization - QT-interval (QT) - we performed a genomewide SNP association study using 100k arrays. QT is normally distributed with heritability >30% and - if unregular - predisposes to sudden cardiac death. Some of its QTLs from candidate gene display strong interaction with gender. QTc_RAS is QT corrected for covariates heart rate, age and gender.
Method: From the population-based KORA S4 survey (n=3,966 after exclusion criteria, including n=2,007 women) 103/103 women were selected from QTc_RAS distribution extremes (below 7.5th and above 92.5th percentile) - to avoid gender confounding - and were genotyped for 88.500 SNPs with CR>0.85 and MAF>0.025 (phase I). 60 most significant SNPs (p<1e-4) were genotyped in additional 200/200 women (phase II). 7 SNPs significant at (p<5e-3) in combined phase I and II where screened in the remaining n=3,366 of S4 (phase III).
Results: Only one SNP in the 5’ region of the CAPON/NOS1AP

gene clearly exceeded the genomewide significance level (p<1e-7). We successfully replicated that association in n=2,646 independent individuals from KORA F3 follow up of survey S3 (p<1e-11).
Conclusions: We identified a QTL of QTc_RAS in the CAPON/NOS1AP gene. The QTL modulates QT interval by about +6 ms and explains 1.5% of trait variance. Future replication attempts as well as interaction analyses will depend on large datasets. Unravelling the genuine architecture of human QTLs will help to devise even more efficient strategies to confirm entire spectra of QTLs for any given human quantitative trait.

C02. Mutations in the facilitative glucose transporter GLUT10 alter arterial patterning and cause Arterial Tortuosity Syndrome

P. J. Coucke[1], *A. Willaert*[1], *M. W. Wessels*[2], *B. Callewaert*[1], *N. Zoppi*[3], *J. De Backer*[1], *J. E. Fox*[4], *G. M. S. Mancini*[2], *M. Kambouris*[5], *R. Gardella*[3], *F. Facchetti*[6], *P. J. Willems*[7], *R. Forsyth*[8], *H. C. Dietz*[9], *S. Barlati*[3], *M. Colombi*[3], *B. Loeys*[1], *A. De Paepe*[1];
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Heritable connective tissue disorders comprise a diverse group of genetic conditions associated with molecular defects involving structural components of the collagen and elastic fibers. A subset of these conditions presents with arterial aneurysms, a major cause of cardiovascular morbidity and mortality. Arterial tortuosity syndrome (ATS) is an autosomal recessive connective tissue disorder characterised by arterial tortuosity, elongation, stenosis and aneurysm formation of the major arteries due to disruption of elastic fibres in the medial layer of the arterial wall. Using homozygosity mapping a candidate gene was mapped in a 4.1 Mb region on chromosome 20q13.1. Haplotype sharing between families originating from the same geographical region narrowed the candidate region to 1.2 Mb containing 7 genes. Mutations in one of these genes, *SLC2A10*, encoding the facilitative glucose transporter GLUT10, were identified in 6 ATS families. We found that GLUT10 deficiency is associated with upregulation of the TGFß pathway in the arterial wall, a finding also observed in Loeys-Dietz syndrome, in which aortic aneurysms associate with arterial tortuosity. The identification of a glucose transporter gene responsible for altered arterial morphogenesis is novel and surprising in view of the previously suggested link of GLUT10 with type 2 diabetes. Our data might shed new light on the mechanisms causing micro-angiopathic changes associated with diabetes and suggest that therapeutic compounds intervening with TGFß signaling represent a new treatment strategy.

C03. An extended consanguineous BBS family with two mutant genes, 3 mutations and no triallelism allows identification of a novel major BBS gene

H. J. Dollfus[1], *V. Laurier*[2], *J. Muller*[3], *C. Stoetzel*[1], *N. Salem*[4], *E. Chouery*[1], *S. Corbani*[4], *N. Jalk*[4], *E. E. Davis*[5], *S. Rix*[6], *J. Badano*[5], *C. Leitch*[7], *C. Leitch*[7], *A. Verloes*[8], *P. Beales*[6], *O. Poch*[3], *D. Bonneau*[9], *A. Mégarbané*[4], *N. Katsanis*[5], *J. Mandel*[3];
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The phenotype of Bardet-Biedl syndrome (BBS) is defined by the association of retinitis pigmentosa, obesity, polydactyly, hypogenitalism, renal disease and cognitive impairment. The significant genetic heterogeneity of this condition is supported by the identification, initially by classical linkage analysis and more recently by comparative genomics, of nine genes (*BBS1* to *9*) implicated in cilia assembly or function.
A large consanguineous Lebanese family living in a small village with 9 individuals affected with Bardet-Biedl syndrome was studied. A classical linkage analysis combined to a SNP homozygosity mapping analysis identified a complex pattern of mutations in BBS genes. In one sibship of the pedigree a *BBS2* homozygous mutation was identified and in three other sibships of the same family a mutation was identified in a novel BBS gene. A last sibship with only one affected individual disclosed two mutations in the new gene. The analysis of this family challenged traditional and SNP linkage because of two loci implicated, three mutations identified and to date no triallelism.
Unlike other BBS genes , the new gene is vertebrate specific and would have been missed by comparative genomics approaches that were successful for other BBS genes. This gene is mutated in at least 20 % of families , a level similar to the BBS1 gene and much higher than for the other known BBS genes, with immediate diagnostic consequences.

C04. A genome-wide SNP association study of rheumatoid arthritis (RA) validates the DNA pooling approach

J. S. Lanchbury[1], *A. Gutin*[1], *V. Abkevich*[1], *T. Merriman*[2], *S. Steer*[3], *K. Timms*[1], *T. Tran*[1], *D. Shattuck*[1], *M. Skolnick*[1];
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The human genome can now be screened for 500,000 or more SNPs using microarray-based technologies. Cost is a major barrier to widespread application of these tools for complex-disease genetic association studies. We developed a robust, cost-effective DNA pooling approach to genome-wide SNP analysis using the Affymetrix Centurion and Illumina Infinium microarrays and tested whether we could detect the association signals for HLA and PTPN22 loci in two RA populations.
Power to detect an association depends strongly on precise measurement of the hybridization intensities and the corresponding allele frequencies. We developed a new approach to estimate allele frequency with high precision. Using this approach the allele frequency for a typical SNP on Affymetrix Centurion and Illumina Infinium microarrays can be estimated with standard deviation of 2.0-2.5%.
We compared power to detect association using our DNA pooling approach with power to detect association based on individual genotyping. The difference between the two approaches was 2% in a modeled example which will be presented.
We hybridized pooled DNA from RA patients and appropriate controls from New Zealand and United Kingdom. Using a strategy that evaluates consistency of association signal across the two populations, SNPs close to HLA-DRA and PTPN22 showed significant evidence of genome-wide association. The combination of random (Affymetrix) and gene-targeted (Illumina) sets of SNP markers offers an enhanced opportunity for genome-wide discovery. The minimal loss of power using the DNA pooling approach facilitates the widespread use of high-content SNP microarrays for a variety of disease association and population genetics applications.

C05. Copy Number Variation of *NCF1* Gene is associated with Rheumatoid Arthritis but not with Psoriatic Arthritis

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OBJECTIVE: Loss of *NCF1* gene, encoding one of the subunits of the NADPH oxidase complex, occurs due to unequal crossing-over with one of the two neighbouring pseudogenes in some patients with autosomal-recessive chronic granulomatous disease (CGD). In a rat model of arthritis a missense polymorphism of *Ncf1* was associated with arthritis severity and shown to reduce the oxidative burst of the NADPH complex. We hypothesized that lower copy number of *NCF1*

might be a susceptibility factor for arthritic disease.
METHODS: To determine copy number variation we developed a quantitative PCR multiplex assay based on Taqman technology. Three cohorts - 199 rheumatoid arthritis (RA) patients, 375 psoriatic arthritis patients (PSA), 282 controls - were screened.
RESULTS: Performance was robust in the majority of DNAs, but as in previous quantitative methods DNA quality was critical. Interestingly, copy number varied between 1-5 gene copies. In all cohorts we observed a higher frequency of heterozygous deletion carriers (1.1-9.1 %) than expected from previously reported estimations of heterozygous carriers of CGD (0.2 % or 1:500). Fisher's exact test revealed a highly significant difference between RA patients and controls: 9.1 % vs. 1.1 % ($p < 4.9 \times 10^{-5}$), while frequency of *NCF1* deletion carriers in PSA patients was similar to controls.
CONCLUSIONS: Our observation of reduced *NCF1* copy number as a susceptibility factor for RA is based on relatively small patient numbers and therefore needs independent confirmation. If confirmed our findings provide evidence of an involvement of NADPH oxidase in the pathogenesis of RA.

C06. Breaking loops for linkage analysis in complex pedigrees

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Recurrent inbreeding is a common feature in various populations, including isolated and immigrant populations resulting in pedigree loops and high frequency of recessive diseases. Likelihood-based linkage analysis is a conventional approach to genetic mapping. The Elston-Stewart algorithm used for complex pedigrees, however, is sensitive to the presence of pedigree loops. In case of multiple loops, approximate methods are to be used. One of them is based on breaking loops by coping certain individuals and conditioning of likelihood of zero-loop pedigree on phenotypes of copied individuals. The selection of breakers is usually based on the number of their possible genotypes. This approach is not effective when many members of pedigree are unobserved or when large number of loci are analysed.
We propose to select the loop breakers on the basis of loss of pedigree informativity induced by loop breaking. We used the classical graph-theory Kruskal approach for selection of optimal loop breakers. In our method a pedigree is represented as a graph with edges wich correspond to the people who are the members of two nuclear families and thus connect them. The weight of each edge is defined based on the loss of pedigree informativity as measured by mean relationship after the elimination of this edge from the graph. We estimated the loss of pedigree informativity in a series of inbred families with hundreds of loops under different algorithms of loop breaking and demonstrated that our algorithm provides minimal loss of information. We implemented our method in a software LOOP_EDGE (http://mga.bionet.nsc.ru/nlru/).

C07. Genome-wide screen for late onset Alzheimer's disease in a complex pedigree from a genetically isolated population

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We studied late onset Alzheimer's disease (LOAD) in a young, genetically isolated population from the Southwest of the Netherlands. Extensive genealogy was collected, resulting in a complex pedigree containing 103 LOAD patients and their 4,645 relatives. This genealogy provides a unique opportunity to identify LOAD loci. However, utilization of such a pedigree in multipoint linkage analysis is computationally challenging. We aimed to develop a new algorithm to cut the entire pedigree into computable sub-units and conduct linkage analysis of LOAD. The algorithm developed clustered all patients into 35 sub-pedigrees, which accounted for 87.5% of the complete genealogic information. Subsequently, we performed a genome-wide search under a dominant model using age dependent penetrances. We confirmed four known loci on chromosomes 1 (LOD = 4.2 between D1S498 and D1S484), 6 (LOD = 2.9 between D6S1610 and D6S257), 10 (LOD = 2.6 between D10S1686 and D10S185) and 12 (LOD = 1.7 between D12S345 and D12S85). We also identified two novel loci on chromosome 3 (LOD = 4.3 at D3S1569) and 7 (LOD = 3.2 between D7S484 and D7S510). These linkage signals are currently under further investigation by fine mapping. In conclusion, we developed an algorithm that can split large pedigrees into manageable sub-units and preserves maximum information about relationships. The ability to identify known loci in the isolated population provides a strong proof of principle. Furthermore, we suggest two novel LOAD loci on chromosomes 3q23 and 7p13.

C08. Genetic background of neurocognitive traits in schizophrenia and bipolar disorder - an association study in twins

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Susceptibility to schizophrenia (SZ) and bipolar disorder (BPD) is influenced by numerous genes affecting a variety of brain functions. Some of these may be inherited in a predisposing configuration without resulting in a clinical phenotype, however affecting the function of the relevant brain systems. Twins represent ideal study subjects to address the genetic background of neurobehavioral traits disturbed in SZ and BPD as they share the same environment during the fetal period and early years of their lives. We tested several variables measuring neurocognitive functions in a Finnish twin study sample consisting of 59 SZ twin pairs (8 concordant, 51 discordant), 20 BPD twin pairs (5 concordant, 15 discordant) and 62 control twin pairs matched for age, sex and demographics. We analyzed 27 SNPs in three candidate genes reported to be associated with SZ: *NRG1*, *DTNBP1*, and *AKT1*. We used linear regression with age, gender, presence of psychosis, zygosity, and the co-twin being affected as covariates. Among the tested neuropsychological variables, four related to verbal learning and memory showed association with SNPs of *AKT1* ($p<.006$). Suggestive association was also observed for various memory functions and a SNP of *DTNBP1* ($p<.01$). The associated traits are known to be affected in SZ and BPD and our study further highlights the importance of quantitative neurocognitive features as endophenotypes for these diseases. Association with *AKT1* and *DTNBP1* also implies the role of these genes in the etiopathogenesis of psychotic diseases and even tentatively elucidates the molecular pathways affected in the disease processes of CNS.

C09. Differential liabilities of coding and non-coding mutations at a major locus in complex disease : *RET* in Hirschsprung disease

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Hirschsprung disease (HSCR) is a multigenic congenital malformation characterized by absence of enteric ganglia in the gut where the *RET* gene plays a central role. High-penetrance *RET* coding sequence (CDS) mutations occur in ~50% of familial HSCR although genetic linkage studies suggest involvement in most families. The penetrance of CDS mutation is sex-dependant. To determine whether RET non-

coding sequences (NCDS) mutations contribute to risk, we studied 876 HSCR patients and their parents from three continents. A common, disease-associated haplotype was constructed for *RET* region in all populations. The largest contribution to risk was made by an enhancer mutation in intron 1. HSCR risk varies by segment length affected (S/L, short/long; TCA, Total Colonic Aganglionosis), gender, and the finding of CDS mutations. We observed that the NCDS mutation makes a greater contribution to susceptibility for S-HSCR and more so in boys than girls. We suggest a model in which the enhancer mutation contributes more to disease liability in the most frequent forms of the disease (i.e. male, S-HSCR, CDS mutation negative) and the rare CDS mutations make the greatest contributions to the less frequent manifestations of HSCR (i.e. female, TCA). The types and parental origin of mutation is also skewed. It is tempting to speculate that the variable prevalence of HSCR across different ethnic backgrounds results from the variable frequency of the disease-associated haplotype. Our data provide a consistent explanation of the genetic features of a disease that stands as a model for multigenic inheritance.

C10. Acrolaryngeal dysplasia: a distinct autosomal dominant acromelic syndrome.

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Acrolaryngeal dysplasia, formally known as “Tattoo” or “Fantasy Island” syndrome, is a distinct autosomal dominant disorder. Through the International Skeletal Dysplasia Registry over 20 cases of individuals originally classified with other acromelic disorders were found to have a characteristic constellation of findings. Affected individuals are usually normal at birth with grow delay becoming evident in the first year of life, progressing to significant short-limbed short stature, most severe in the hands and feet. They develop a distinct hoarse voice, down slanting palpebral fissures, thickened eyelashes and/or dictachiasis, striking muscular habitus, very small hands with limited flexion and in some cases, significant asthma and restrictive pulmonary disease. Radiographic findings include a relatively normal axial skeleton, relatively small capital femoral epiphyses with a medial downward slant and significant brachydactyly. The metacarpals are primarily involved with small pointed epiphyses, and a “carved out” appearance of proximal metacarpals 2-5. Two instances of vertical transmission were observed, the remainder of the cases being sporadic, suggesting autosomal dominant inheritance. While the clinical phenotype resembles geleophysic and acromicric dysplasia, the radiographic findings in the metacarpals and hips are different and in neither disorder is the distinct voice and typical muscular habitus seen. Geleophysic dysplasia has been chararcterized as a lysosomal storage disorder, whereas there is no lysosomal vacuolization in acrolaryngeal or acromicric dysplasia. The distinct irregular laryngeal nodules of chondroid metaplasia and the bony metaplasia of the tracheal and bronchial cartilage observed in acrolaryngeal dysplasia may provide a clue as to the pathogenesis of this disorder.

C11. Achalasia, megacolon, skeletal deformities associated with generalized angiodysplasia and severe growth retardation, low copper, ceruloplasmin and zinc levels in two distinct consanguineous families. A new autosomal recessive condition.

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The aetiology of achalasia is largely unknown although a gene has been identified for adrenal insufficiency, achalasia and alacrima syndrome (Triple A syndrome, MIM # 231550). Familial occurance (MIM # 200400) is extremely rare and achalasia in those cases is usually seen as an isolated abnormality. We report an undescribed associated achalasia in two sisters and two brothers in distinct families ascertained from different geographical regions. The major findings are: achalasia, megacolon, skeletal changes such as, triangular facial face, high palate, kyphoscoliosis, finger deformities and nail dysplasia as well as severe growth retardation. These cases also had generalized angiodysplasia, low serum copper, ceruloplasmin and zinc levels. The intelligence was normal. No abnormality was detected in cytogenetic analysis. Minimal variation in the phenotype was observed within and between the families. To the best of our knowledge this striking association with achalasia has not been reported. Consanguineous nature of both families suggested autosomal recessive inheritance. For the gene symbol, we suggest AAMS (**A**chalasia, **A**ngiodysplasia, **M**egacolon, **S**keletal changes). No additional abnormalities including malignancy, multiple endocrinopathies etc., have been demonstrated in ten years folow-up of these cases.

C12. Holoprosencephaly: clinical and genetic study about 340 patients (1996-2006)

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Holoprosencephaly (HPE) is the most common brain malformation resulting from incomplete cleavage of the prosencephalon (1 out of 16.000 live births; 1 out of 250 conceptuses). HPE is associated with a wide spectrum of craniofacial malformations ranging from lethal forms (alobar HPE with cyclopia) to less severe forms such as lobar HPE and normal face.

The aetiology is very heterogeneous involving environnemental factors, chromosomal abnormalities and at least 7 genes.

Since 1996, our team has started to work on the wide clinical and genetic variability of holoprosencephaly. Patient (foetuses or children with normal karyotype) samples and clinical data (from HPE to microforms) were collected from medical teams in France and Europe. Systematic mutation analysis and genomic rearrangements (QMPSF, MLPA) screening of the four main genes (*SHH, ZIC2, SIX3, TGIF*) were performed. 71 sequence changes were identified among 340 DNA samples at a rate of about 20%.

27 familial cases confirmed extreme clinical variability. 9 patients samples with 2 microdeletions were identified supporting multiple-hit hypothesis involving others genetics and/or environnemental factors. As genetic counselling must be done carefully, we performed molecular prenatal diagnosis fours times with fetal US scan and cerebral MRI screening.

At last, we show involvement of cerebral malformations as Aprosencephaly/Atelencephaly is linked to *SIX3* mutations and cerebellar hypoplasia to *SHH* gene. This work has improved molecular diagnosis’ rate in Holoprosencephaly and therefore the genetic counselling.

C13. *FBN1* mutations in patients with incomplete Ghent criteria in a series of 1057 probands: Further delineation of type I fibrillinopathies

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Mutations in the *FBN1* gene cause Marfan syndrome (MFS) and type I fibrillinopathies. The proportion and the clinical characteristics of patients carrying a *FBN1* mutation with incomplete Ghent criteria remains to be determined. From a large international collaborative study including 1057 probands with a *FBN1* mutation, 30% did not fulfill the Ghent nosology if the presence of a *FBN1* mutation was not considered as a major criterion. Since some features of MFS appear in adulthood, we analyzed a subgroup of 172 adult probands

with incomplete Ghent criteria (16%). The mean age at last follow-up was 36 years. The majority of patients (78/172) had one major and at least one minor system involvement, 17/172 had 2 major systems involvement, 46/172 had an isolated major system involvement [isolated aortic dilatation (n=22), isolated ectopia lentis (n=13), isolated skeletal manifestations (n=9), isolated mitral valve prolapse (n=2)] and 31/172 had one to four minor systems involvement. *FBN1* mutations were preferentially located in the 5' region of the gene in patients with isolated ectopia lentis and isolated aortic dilatation and in the 3' end of the gene in patients with isolated skeletal manifestations. No PTC mutation was found in association with isolated ectopia lentis. The molecular results allowed reclassification in 55% of this subpopulation as classical MFS according to Ghent criteria, giving emphasis on the indication of a *FBN1* mutation study in patients with two major or one major and at least one minor system involvement in order to identify individuals at risk for aortic dilatation.

C14. Non congenital paediatric Myotonic Dystrophy: clinical and genetic study in a series of 44 patients

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There are 2 paediatric forms of myotonic dystrophy type 1 : the well-known severe congenital form and the more recently described childhood-onset type. Nevertheless, the nomenclature of DM1 in children remains confusing.

We report a series of 44 patients (20 females and 24 males) aged from 6 to 18 years, presenting with a paediatric non congenital form of DM1, in order to precise its clinical and genetic characteristics. The results showed that :

1. The main clinical symptoms were slowness, hypersomnia, tiredness, dysarthria, and learning difficulties. At clinical exam, facial involvement and myotonia were frequent, but usually not recognized by the patients.

2. School disability was the most important feature in daily life, whereas mental retardation was present in 55% of the patients

3. 16/44 patients had mild symptoms before one year of age, leading to identify an "early childhood form" of DM1

4. The sex ratio of transmitting parents was nearly one (24/20)

5. Molecular genetic studies showed CTG repeat size ranging from 200 to 1800 (mean : 679), while CTG repeat size from transmitting parent was from 65 to 1100 (mean : 380)

This report, the most important series of patients with paediatric non congenital form of DM1, confirms (1) the existence of a clinical continuum in paediatric forms of DM1 and (2) the presence of non muscular symptoms mainly learning difficulties. Several groups of these patients have been extensively studied for (1) cognitive profile (n=36), (2) reading and spelling impairments (n=23), (3) sleep evaluation (n=20) and will be presented.

C15. The origin of EFNB1 mutations in craniofrontonasal syndrome: frequent somatic mosaicism and explanation of the paucity of carrier males

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Craniofrontonasal syndrome (CFNS) is an X-linked disorder in which males, despite being less severely affected than females, are paradoxically under-represented in pedigrees. To understand the molecular basis for this, we have examined the EFNB1 mutations in 59 CFNS families (39 new, 20 previously published). We identified 27 novel intragenic mutations and 3 deletions, 2 of which completely delete EFNB1. All mutations support a partial or complete loss-of-function mechanism. A key goal of the study was to define the parental origin of germline mutations. As mosaicism would confound this analysis we first screened samples using Wave-DHPLC, specific restriction digests and Pyrosequencing. Mosaicism was identified in 6 cases (out of 53 informative for this study), including in an apparently unaffected parent with 2 affected children, a sporadic case, and an affected female with two unaffected children. The proportion of mutant allele was quantified by Pyrosequencing and varied widely in different subjects and tissues (2-48%). In the remaining samples a paternal origin of mutation was demonstrated in 13 out of 15 informative cases. The bias towards mutations arising in the paternal germline is probably the major determinant of the scarcity of males in CFNS pedigrees, as only affected females are produced in the first generation. Post-zygotic mutations, the reduced reproductive fitness of affected females, and the occurrence of two thirds of X chromosomes in females also contribute. Our results have important implications for genetic counselling in CFNS: the possible origins of mutation must be taken into account when providing recurrence risks.

C16. *Amisyn*, *SCAMP5*, *CLIC4* and *NBEA* are candidate genes for autism and suggest a role for neuron vesicle trafficking in the pathogenesis of autism.

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Autism is a neurodevelopmental disorder of unknown cause and pathogenesis. At the ESHG 2004 meeting, we reported on the identification of three different genes in three unrelated persons with idiopathic, non-familial autism carrying a *de novo* chromosomal translocation: *NBEA*, *CLIC4* and *amisyn*. Interestingly, the corresponding proteins of all genes have a possible function in vesicle trafficking in the brain.

Recently, in the *CLIC4* patient, also *SCAMP5* was found to be affected. SCAMP5 is the brain-specific member of the 'secretory carrier membrane protein' family involved in vesicle budding from *trans*-Golgi.

We studied the involvement of all these genes in the regulated secretory pathway of dense-core secretory granules (counterparts of neuronal large dense-core vesicles) by overexpression versus RNAi-mediated gene knockdown in the mouse beta-TC3 neuroendocrine cell line. Overexpression of amisyn and SCAMP5 result in a significant decrease of secretion. Interestingly, knockdown of NBEA, SCAMP5 and amisyn result in a significant increase of secretion. However, we showed no involvement for CLIC4 in regulated secretion. In conclusion, these *in vitro* data suggest a role for NBEA, amisyn and SCAMP5 as negative regulators of neuron vesicle trafficking and/or fusion.

In addition, preliminary *in vivo* data on the blood platelets of these patients revealed specific abnormalities in both morphology and secretion of the vesicles.

Taken together, the identification of genes affected by translocations in patients with autism in combination with the *in vitro* and *in vivo*

functional data concerning the corresponding proteins implies that vesicle trafficking in neurons may be involved in the pathogenesis of autism.

C17. Disrupted function and axonal distribution of mutant tyrosyl-tRNA synthetase in dominant intermediate Charcot-Marie-Tooth neuropathy

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Charcot-Marie-Tooth neuropathies (CMT) are common disorders of the peripheral nervous system caused by demyelination or axonal degeneration, or a combination of both features. We previously assigned a locus for an autosomal dominant intermediate CMT type to chromosome 1p34-p35 (DI-CMTC). Here we identified two heterozygous missense mutations and one *de novo* deletion in tyrosyl-tRNA synthetase (YARS) in three unrelated DI-CMTC families. Biochemical experiments and genetic complementation in yeast demonstrated partial loss of aminoacylation activity of the mutant proteins, and mutant YARS, or its yeast orthologue TYS1, reduced yeast growth. YARS localizes in axonal termini of differentiating primary motoneuron and neuroblastoma cultures. This specific distribution was significantly reduced in cells expressing mutant YARS proteins. YARS is the second aminoacyl-tRNA synthetase found to be involved in CMT, linking protein-synthesizing complexes with neurodegeneration.

C18. Distinguishing neurodegenerative disorders with tau pathology using mRNA expression microarrays

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Despite intensive research, the aetiology of Alzheimer's disease (AD), Pick's disease (PiD), Progressive Supranuclear Palsy (PSP) and Frontotemporal dementia (FTD) is still largely unknown. Due to the overlap between these diseases clinical diagnosis of these patients is difficult. Even on post-mortem material classification of disease type is labour intensive and complex. Microarrays can provide insight in the complexity and relationships between diseases and normal aging, as they provide data of the simultaneous activity of multiple genes and cellular pathways.

To investigate whether microarrays are an ideal platform for disease classification, we analysed snap frozen post-mortem tissue from the medial temporal lobe of pathology confirmed patients with four different tauopathies AD, PSP, PiD, FTD and control subjects for gene expression. To exclude as much non-disease-related variation all patients were age, gender, APOE-ε and MAPT (tau) haplotype matched.

Using several rounds of analysis we identified a set of 166 genes that can discriminate all patient groups from controls. Furthermore PSP and AD and FTD/PiD also clustered into separate clusters. These findings are a first step towards the development of an accurate microarray-based classification test.

C19. A mutation in the *nup62* gene causes Infantile Bilateral Striatal Necrosis

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Autosomal recessive Infantile Bilateral Striatal Necrosis is characterized clinically by developmental arrest at the age of one year, dysphagia, choreoathetosis, pendular nystagmus and progressive degeneration of the basal ganglia. We have mapped the disease gene in the candidate region to ~230 kb on 19q13.33 in six interrelated Bedouin families including in total 10 patients and 28 unaffected individuals. Sequencing of the *nup62* gene revealed a missense mutation causing a change from glutamine to proline (Q391P) in exon 3 in all the patients, producing a substitution from a polar, hydrophilic residue to a non-polar, neutral residue. All the other 12 candidate genes were sequenced, and no pathogenic sequence changes found. The mutation was not present in 400 control chromosomes from ethnically unrelated individuals; it was found only in the heterozygous state in 12 out of 280 chromosomes in the Bedouin controls living in the same geographic area as the original families, as expected due to the high frequency of carriers within this population. Four new patients in two Bedouin IBSN families identified recently were homozygous for the same *nup62* mutation. Comparisons of p62 protein sequences from diverse species indicate that glutamine at position 391 is highly conserved. This is the second example of a nuclear pore complex protein causing mendelian disease in humans (the first is triple A syndrome). Both diseases share central nervous system involvement. Our findings suggest that p62 has a cell type-specific role and is important in the degeneration of the basal ganglia in humans.

C20. Impaired ganglioside synthesis involved with pathogenic mechanism in a familial form of multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) characterized by features including demyelination, inflammation and degeneration of central axons. Genetic and non-genetic factors both have roles in the susceptibility to MS. Here we present evidence of a change in ganglioside synthesis as a foundation of the disease in a pedigree in which MS segregates. We previously demonstrated a linkage between the MS phenotype and markers on 12p12 conditional on the presence of HLA DR15, DQ6 alleles in a pedigree of Pennsylvania Dutch extraction. A candidate locus encompassing 18 cM segment of 12p12 was analyzed for candidate gene analysis. Direct sequence of the disialoganglioside 3 synthase (GD3S) gene localized in that region show a novel G29A genetic variant present in the 5' splice donor site of intron 4/5 of the GD3S gene (CMP-NeuAc:NeuAc alpha 2-3Gal beta 1-4Glc beta 1-1'Cer alpha 2,8-sialyltransferase). RT-PCR revealed that in MS patients there is a premature degradation of the GD3S mRNA molecules. Additionally real-time quantitative RT-PCR assays show that the GD3S transcript is about 150 times lower when compared to the normal sample. Moreover immunofluorescence analysis on peripheral blood leukocytes (PBLs) and immunoprecipitation (IP) western blots of the protein extract from the same cells of MS patients confirm an altered staining pattern when compared with controls. The discovery of this specific molecular change associated with MS may shed light on the pathogenic mechanism in the disease.

C21. Transcriptional deregulation by the mutant Huntington Disease (HD) protein: implications for pathogenesis

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Huntington's disease (HD) is caused by the expansion of a polyglutamine repeat and can be used as a model to study neurodegenerative disorders caused by aggregation-prone proteins. It had been proposed that the entrapment of transcription factors (TFs) in protein aggregates plays an important role in HD pathogenesis. In this study we studied the highly-related co-activators and Histone Acetyltransferases CBP and p300 in an established HD-exon1 (httex1p) PC12 cell model. The results indicate that mutant htt specifically affects the activity of CBP, and not p300, via different mechanisms, including protein binding and protein degradation. Our data challenge the model that interference of mutant htt with TFs is caused primarily by their entrapment in aggregates. To get more insight in the underlying mechanisms, we performed biochemical comparison studies of httex1p. The results showed that soluble wild type httex1p fragments are predominantly present in higher molecular weight complexes, while their mutant counterparts are mainly present in monomeric form. These findings suggest that especially an increased amount of soluble monomeric forms of mutant httex1p may facilitate aberrant interactions both with itself via the polyglutamine stretch and with other proteins, such as CBP and thereby contribute to molecular pathogenesis.

C22. Defective oxidative phosphorylation in thyroid oncocytoma is associated with pathogenic mitochondrial DNA mutations affecting complexes I and III.

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Cells with an aberrant accumulation of mitochondria characterize oncocytic tumors. These tumors commonly arise in the thyroid and their pathogenesis is unknown. In order to assess mitochondrial function in neoplastic oncocytic cells, we studied the thyroid oncocytic cell line XTC.UC1 and compared it with other thyroid non-oncocytic cells. Only XTC.UC1 were unable to survive in conditions forcing cells to rely only on mitochondria for energy production. The rate of respiration and ATP synthesis driven by complex I substrates was severely reduced in XTC.UC1 and the enzymatic activity of complexes I and III dramatically decreased, in conjunction with an enhanced production of reactive oxygen species. Transmitochondrial cell hybrids (cybrids) carrying XTC.UC1 mitochondrial DNA (mtDNA) were generated to discriminate whether the energetic failure depended on mitochondrial or nuclear DNA mutations. XTC.UC1 cybrid clones showed a reduced viability and ATP content similar to that of the parental XTC.UC1, pointing to the existence of mtDNA alterations. Sequencing of XTC.UC1 mtDNA identified a mutation generating a premature stop codon in ND1 (complex I) and a missense substitution in the catalytic site of cytochrome b (complex III).
Our work provides the first demonstration that impaired mitochondrial function of XTC.UC1 is due to a combined complex I/III defect associated with mtDNA mutations as proven by the transfer of the biochemical deficiency of the mutant mtDNA into the cybrids. These mutations may represent valuable biomarkers for thyroid oncocytic tumors and contribute to the development of specific treatments for the cure of this form of cancer.

C23. Aggressive fibromatosis, pathways that cross-talk with Wnt signaling

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Desmoid tumors, also known as aggressive fibromatosis, are rare fibroblastic tumors that exhibit a wide range of local aggressiveness, from largely indolent to locally destructive. Although, we have already shown constitutive activation of Wnt pathway in Desmoid tumors due to APC or B-Catenin mutation, our recent data revealed involvement of some other signaling pathways like TGF/BMP signaling pathways in pathogenesis of Desmoid tumors. Western blot analysis revealed over expression of phospho-smad2 in desmoids in compare to control tissue (Fascia) of same patient. Induction of TGF-signaling pathway in normal human fibroblast, increased total and active form of Beta-Catenin and consequently increased proliferation in these cells. Over-induction or blocking of TGF-B signaling pathway in Desmoid cells changed their behavior particularly BrdU incorporation .Using transfection assay, TGF-B could significantly down-regulate IGFBP6 promoter (One of the direct target genes of Wnt-B-Catenin which is highly down-regulated in Desmoid tumors) in different cell line, while it could up-regulate TOPflash activity. The cross-talk between these pathways leads us to a proposed Vogel-gram for Desmoid tumors.

C24. Genome-wide analysis to unravel the molecular mechanisms behind vitamin D resistance

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The role of vitamin D (1,25-dihydroxyvitamin D_3) in cancer was suggested by a strong epidemiological association between prostate, breast, and colon cancer and vitamin D deficiency, and is thought to be a consequence of its anti-proliferative action.
We have initiated studies where we employ genomic approaches to unveil the genetics behind the anti-tumorigenic action of vitamin D. In our studies, we made use of the breast tumor cell line MCF-7 of which four independent biological vitamin D resistant replicates were produced. Vitamin D resistance was studied with a variety of genomic techniques; including expression arrays, NMD arrays and oligo CGH arrays. We, and others, have shown that studying drug resistance using different array techniques may point directly towards pivotal genes underlying cancer progression. For the experiments RNA and DNA from all 4 resistant cell lines was hybridized directly with the respective RNA/DNA of the parental cell line onto human oligonucleotide (60-mer) arrays of 30.000 probes.
The oligo array CGH experiments pointed to an area in chromosome 11q as candidate region of DNA copy number changes. Parallel expression analysis on the 30K oligo arrays revealed that no single significant gene was involved in the acquired resistance. Instead a set of genes was consistently altered, which led us to employ pathway analysis in the search for common signatures. The deregulation of the pathway involving EGFR (epithelium growth factor receptor) was identified in this analysis, indicating an important role for the EGFR pathway in the anti-tumorigenic effect of vitamin D.

C25. The major epigenetic effect of the histone deacetylase inhibitor butyrate is a paradoxical decrease in promoter histone acetylation and decrease in gene activity.

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Butyrate is a histone deacetylase inhibitor (HDACi) and chemical derivatives are used in clinical trials. It is structurally similar to valproic acid and phenylbutyrate that are approved for treatment of epilepsy and cancer. HDACi treatment increases the overall acetylation of histones, which is supposed to reactivate epigenetically silenced genes. The liver cell-line HepG2 was treated for 12h with sodium-butyrate, which dramatically increased the total levels of acetylated H3 and H4. Distribution of AcH3 and AcH4 was characterised by ChIP-chip in 1% of the human genome as defined in the ENCODE project. The major

and surprising finding was that 100-150 regions with high AcH3 and AcH4 suffered a pronounced deacetylation after treatment, many of them located around transcription start sites. ChIP and locus specific PCR was performed on a time series and confirmed 11/11 analysed regions. For each of these promoters the expression of the gene was analysed and was found to be down-regulated. ChIP-chip analysis of H3 and H3K4me showed that nucleosome loss could not explain the results. ChIP of RNA PolII suggested that transcription initiation and/or elongation may be affected. Immunofluorescence analysis showed that the increase in total histone acetylation was localized to the nuclear periphery and it may be located in heterochromatic regions. It is probable that also other HDACi's may have this effect on histone acetylation in euchromatic regions. Our results indicate that they should be analysed in a similar way and that pre-assumptions about their molecular mechanisms of action might need to be revised.

C26. Birt-Hogg-Dube : A syndrome the geneticist should know

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In our full-service genetics consultation we see approximately 200 families/year for assessment of a hereditary predisposition to cancer. Most consultations concern breast /ovarian or colorectal cancers, and most gene testing comes back negative. Although textbooks suggests that "rare cancer-predisposition syndromes" account for less than one percent of familial aggregation of cancer, our experience suggests that syndromic entities may well be under-diagnosed. One such condition is the Birt-Hogg-Dube (BHD) syndrome, due to mutations in the presumed tumor-suppressor gene folliculin (FCN) on chromosome 17p. BHD is best known to dermatologists because of skin lesions with characteristic histology: fibrofolliculomas, trichodiscomas and acrochordons. Renal tumors, specifically oncocytomas and papillary renal cell carcinomas, are highly characteristic of BHD, which also predisposes to spontaneous pneumothorax/ pulmonic cysts and to benign and malignant tumors of the uterus, ovaries, breast and colon. We describe here the clinical, genetic and histological features of three families with BHD syndrome presenting to the cancer genetics consultation in the same year. All adults over 30 had typical facial and thoracic skin lesions and each family had at least one person with a benign or malignant kidney tumor. Two families had pneumothorax. We believe that a thorough physical exam and the conscientious histological verification of benign and malignant tumors in individuals attending the cancer genetics clinic, will result in improved detection of this under-diagnosed cancer predisposition syndrome.

C27. Identification of methylation and expression abnormalities associated with breast cancer.

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We have elaborated a novel modification of arbitrary-primed methylation sensitive PCR to identify CpG islands (CGIs) abnormally methylated in breast cancer (BC). By use of this technique we have identified CGIs belonging to *SEMA6B*, *BIN1*, *VCPIP1*, *LAMC3*, *KCNH2*, *RPSA*, *CACNG4* and *PSMF1* genes. Methylation frequencies in 108 BC samples were evaluated by methylation sensitive PCR and/or direct sequencing of bisulphite-treated DNA (table1). By means of bisulphite sequencing we have constructed fine methylation maps for parts of the identified genes' promoter CGIs demonstrating individual cytosine methylation patterns characteristic for each. Methylation mapping revealed nonrandom distribution of methylated cytosines across the CGIs with certain regions within the same island being frequently and some rarely/never methylated in BC. Methylation was not restricted to the core regions of CGIs. Thus, in *BIN1* the intronic part of the promoter CGI showed abnormal methylation, while its core region was never methylated, which had led other authors to an erroneous conclusion of absence of *BIN1* methylation in cancer, which was discordant with the observation of its reactivation after 5-aza-2'-deoxycytidine treatment. Expression patterns were evaluated for every of the genes under study (table1, 16 BC samples versus their normal counterparts). Discussion of the structural/functional features of the CGIs and the patterns of expression of corresponding genes in BC will be presented. *The research is being partially supported by Applied Biosystems, USA.*

Table1. Methylation and expression profiling of the genes identified in the study.

Gene	Locus	Protein	Methylation	Reduced expression	Normal expression	Elevated expression
SEMA6B	19p13.3	Semaphorin 6B	38%	44%	50%	6%
BIN1	2q14.3	Bridging integrator 1	18%	67%	33%	0
VCPIP1	8q13.1	Deubiquitinating protein VCIP135	17%	70%	30%	0
LAMC3	9q34.13	Laminin gamma-3 chain	8%	87%	13%	0
KCNH2	7q36.1	Potassium voltage-gated channel subfamily H member 2	7%	63%	31%	6%
RPSA	3p22.2	40S ribosomal protein	2%	18%	82%	0
CACNG4	17q24.2	Calcium channel, voltage-dependent, gamma subunit 4	3%	37,5%	25%	37,5%
PSMF1	20p13	Proteasome inhibitor subunit 1 (PI31)	3%	69%	31%	0

C28. Loci of shared segmental aneuploidy in the genomes of healthy and mentally retarded subjects detected by Array-CGH

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By genome-wide segmental aneuploidy profiling with a 3,782 BAC array we detected in a panel of 131 unrelated affected children and 21 of their unaffected parents on average 35 autosomal loci of segmental aneuploidy (SA) per subject. In total, we identified 696 distinct loci of SA that were shared among patients and parents. These loci occur as deletion, duplication, or both, in frequencies up to 45% within our study population. Subjects of Turkish and Arab/Berber origin showed significant overlap with Caucasians (87% and 92%, respectively). Plots of the cumulative number of shared loci of SA indicate that their total number may be finite. Loci of SA were distributed in the euchromatin with equal densities among Giemsa-light and Giemsa-dark bands. Out of the 68 most frequently occurring loci of SA, 21 contained sites of segmental duplication on the same chromosome, while 31 and 63 BAC clones were flanked by segmental duplications within a distance of 1 and 2 Mb, respectively. Comparison with a set of 39 randomly chosen BAC clones showed highly significant association of loci of SA with segmental duplications (p values of 0.0001, 0.0012 and <0.0001, respectively). Our data are consistent with the hypothesis that most loci of SA have been generated through non-allelic homologous recombination mediated by intrachromosomal sites of homology. Since loci of SA occur frequently in the general population, they should be taken into account before clinical conclusions are to be drawn upon detection of segmental aneuploidies in patients with congenital abnormalities or mental retardation.

C29. Distribution of recurrent copy number variations in different ethnic populations

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Recent studies have revealed a new type of genetic variation in the human genome encompassing relatively large genomic segments (~100 kb - 2.2 Mb)[1-4]. These variations are referred to as copy number variations (CNVs). The full nature and extent of CNVs in terms of their genomic locations and frequencies in different ethnic populations is still largely unknown. Here we describe the use of MLPA[5] for determining the frequencies of 12 regions of recurrent CNV recently detected by array CGH[3,4]. More than 300 individuals from five different

ethnic populations, including three distinct European, one Asian and one African population, were tested for the occurrence of CNV using at least one sequence-unique MLPA primer pair per genomic region. Seven of these regions showed extensive CNV in all populations, including the β-defensin gene cluster (8p23.1), the *TBC1D3* region (17q12), and (part of) the *NSF* gene (17q21.31). For the *NSF* locus, ~70% of individuals showed copy numbers different from the median, with estimated copy numbers varying between 2 and 7. Furthermore, we detected different copy number distributions of the *NSF* gene between populations, suggesting that this locus is under selective pressure. Future studies will be aimed at the effect of recurrent CNVs on gene expression levels and their potential involvement in human health and disease.

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[2]Fredman *et al.* Nat.Genet. (2004) **36**, 861-866
[3]Iafrate *et al.* Nat.Genet. (2004) **36**, 949-951
[4]De Vries *et al.* Am.J.Hum.Genet. (2005) **77**, 606-616
[5]White *et al.* Hum.Mutat. (2004) **24**, 86-92

C30. Systematic prediction of the boundaries of large copy number variants in the human genome

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Large-scale copy number polymorphisms (CNPs) represent an abundant form of genomic variation in humans (1-3) and are likely to be of increasing importance in molecular studies related to human diversity and disease (4). While existing technologies allow the detection of SNPs and small (<<1kb) deletions and insertions, the precise boundaries of larger CNPs have remained inaccessible. We have developed a combined computational and experimental approach that involves high-resolution oligonucleotide tiling array data processing, and a data mining strategy utilizing custom Gaussian Hidden Markov Models, enabling the systematic discovery of CNP boundaries across the human chromosomes 21, 22, X, and Encode regions. Predicted breakpoints are validated by DNA sequencing following PCR or vectorette PCR amplification. Previously, breakpoint validation for genomic alterations detected on chromosomes 22 and 11 showed that boundaries are predicted with an accuracy <500 bp (ref. 5). We apply the approach on high-resolution comparative genome hybridization data from various patients and healthy individuals, utilizing chromosome-specific oligonucleotide tiling arrays (~400,000 probes) with average probe-spacing <100 bp. Our initial results suggests that the approach will help discriminate disease variants indistinguishable by technologies currently applied in clinics.

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C31. Identification of haplotypes in the human Foxo1a and Foxo3a genes influencing disease at old age and lifespan

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Recently Daf-16 has been shown to regulate lifespan in nematodes and flies. In mammals four Daf-16 homologues are present: Foxo1a, Foxo3a, Foxo4 and Foxo6. Here we present the first data about the influence of human Foxo1a and Foxo3a on disease at old age and lifespan. This study was carried out in the population based Leiden 85-plus Study consisting of 1245 participants aged 85 years and older, with a follow-up of 5489 person-years. Based on HapMap, 3 htSNPs from Foxo1a and 9 htSNPs defining two haplotype blocks from Foxo3a were chosen. In addition 1 SNP from Foxo1a and 2 SNPs from Foxo3a were selected to describe the area before the haplotype block. The haplotype analyses of Foxo1a revealed that carriers of 221 haplotype have higher Hba1C levels compared to 111 carriers (5.90 (0.02) vs. 5.70 (0.01), p=0.025), which was accompanied by a non-significant trend for higher prevalence of myocardial infarction and diabetes. In addition, the 221 carriers had higher all-cause (1.08 (1.00-1.17)), non-cardiovascular (1.11 (1.01-1.22)), and other cause of mortality (1.18 (1.04-1.33)). In block-1 of the Foxo3a, carriers of the 1112 haplotype had higher risk for stroke (1.92 (1.19-3.08)), but lower for myocardial infarction (0.61 (0.37-0.99)) compared to the 1111 haplotype carriers. The same participants had higher all-cause (1.09 (1.00-1.19)) and stroke (1.28 (1.03-1.60)) mortality. No associations with the Foxo3a block-2 or with the SNPs before the haplotype blocks were found. The results of this study suggest that polymorphisms in the Foxo1a and Foxo3a gene have an influence on human lifespan.

C32. Identification and Functional Analysis of CITED2 Mutations in Patients with Congenital Heart Defects

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We present for the first time functionally relevant mutations of *CITED2* in patients with congenital heart defects (CHD) (Sperling et al. Human Mutation 26(6), 575-582, 2005). Recent reports have demonstrated that mice lacking the transcription factor Cited2 die in utero showing various cardiac malformations. *CITED2* encodes a CBREBBP/EP300 interacting transcriptional modulator of HIF1A and TFAP2.

To study the potential impact of sequence variations in *CITED2* for CHD in human, we screened a cohort of 392 well-characterized patients and 192 control individuals using DHPLC, sequencing and Amplifluor™ genotyping techniques. We identified 15 *CITED2* nucleotide alterations, thereof seven alterations that were only found in CHD patients and not detected in controls including three mutations leading to alterations of the amino acid sequence (p.Ser170_Gly178del, p.Gly178_Ser179ins9, p.Ser198_Gly199del). All three of the amino acid changing mutations cluster in the serine-glycine-rich junction of the protein to which so far no functionality had been assigned. Here we show that these mutations significantly reduce the capacity of CITED2 to transrepress HIF1A, additionally the p.Ser170_Gly178del mutation significantly diminishes TFAP2C coactivation. This reveals a modifying role for the serine-glycine-rich region in CITED2 function. In summary, these observed mutations occurring in patients with septal defects indicate the causative impact of *CITED2* in the development of CHD in human.

C33. Mutations in Desmoglein-2 gene are associated to arrhythmogenic right ventricular cardiomyopathy

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Arrhythmogenic right ventricular cardiomyopathy (ARVC) is a myocardial disease characterized by progressive myocyte loss and fibrous and fatty tissue replacement of the right ventricular free wall, which is the substrate for reentrant arrhythmias and sudden death. The primarily mode of inheritance is autosomal dominant with reduced and age-related penetrance. Five disease genes have been identified so far, three of them (desmoplakin, plakophilin-2 and plakoglobin) are involved in the desmosomal complex.

We hypothesized that mutations in desmoglein-2 (DSG2), the only desmoglein isoform expressed in cardiac myocytes, may account for ARVC.

Sixty ARVC probands were screened for DSG2 mutations by denaturing high-performance liquid chromatography (DHPLC) and direct sequencing. Ten DSG2 mutations have been identified in 9 patients (15%). Among the DSG2 mutations, six were missense (Y86C, G100R, N266S, K294E, E331K, V391I), two insertion-deletions (G678fsX681, E418fsX419), one a nonsense (Q558X) and one a splice site mutation (1181-2A>G). None of the detected nucleotide changes was found in 560 control chromosomes. An endomyocardial biopsy was obtained in five patients, showing extensive loss of myocytes with fibro-fatty tissue replacement. In three of them, electron microscopy

investigation was performed, showing intercalated disc paleness, decreased desmosome number and intercellular gap widening.
This is the first demonstration that mutations in DSG2 gene are associated to ARVC. Based upon current data, we confirm that many forms of ARVC are due to alterations in the desmosome complex.

C34. Genotype- Phenotype correlation in Patients with Short stature: Clinical indicators of SHOX Haploinsufficiency

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Short stature affects three in a hundred children. Despite an assumed extraordinary genetic heterogeneity, mutations in a single gene, *SHOX*, which encodes a homeodomain transcription factor involved in bone growth, are relatively frequently found in patients with short stature. Haploinsufficiency of the *SHOX* gene causes short stature with varying phenotypes ranging from isolated short stature to Leri-Weill dyschondrosteosis and Langer dysplasia. In this study, we assessed the association between the genotype and phenotype in a large cohort of short children from 14 different countries with an average age of 7.6 years. Screening of 1641 unrelated children with sporadic or familial short stature revealed *SHOX* mutations in 68 (4.2%) individuals. While mean height was not different between patients with and without *SHOX* deficiency (-2.57 versus -2.58 SDS), detailed anthropometric measurements in all children revealed several bone deformities including short forearm and lower leg, cubitus valgus, Madelung deformity, high-arched palate and muscular hypertrophy that differed significantly ($p<0.001$). These phenotypic data were analysed and compared to 33 children with Turner syndrome where haploinsufficiency of SHOX is thought to be responsible for the height deficit. Different types of *SHOX* mutations (48/70.6% classified as complete deletions, 4/5.9% as partial deletions and 16/23.5% as point mutations) were also compared to the respective phenotype suggesting a tendency towards a more severe phenotype in individuals with homeodomain missense mutations. Altogether, this study offers a detailed genotype-phenotype comparison in a large cohort of children with short stature and provides clear quantitative guidelines as to which children call for testing of the *SHOX* gene.

C35. PTEN Related Disorders - a national clinical study in the UK

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PTEN related disorder is the preferred term encompassing a group of syndromes including Bannayan-Zonana, Riley-Ruvalcaba syndrome (BRR), benign familial macrocephaly and Cowden syndrome. We present a clinical study of 30 individuals from 18 families with a known PTEN mutation. The patients were ascertained through clinical geneticists or via the BRR patient group with the aim of documenting the different clinical presentations dependent on age and deriving information about the national history of the condition.
The age range of the probands was between 2 years and 69 years of age. The commonest presentation in childhood was developmental delay. In adulthood the presentation was more varied and included cerebellar disease and bowel cancer. Pertinent clinical findings of the whole cohort included the observations that all patients were macrocephalic with a head circumference over the 98th centile; four out of thirty had a diagnosis within the autism spectrum, walking was delayed with an average age of 20 months and twelve individuals had required speech therapy. On examination, only one adult had normal skin. Of fourteen adults who have been investigated eleven have benign thyroid disease. There were no cases of malignant thyroid disease, but one case of breast and colon cancer was observed.
In conclusion, we did not demonstrate a genotype-phenotype correlation; variable expression within and between families was seen. Penetrance of the condition is age related. Practical clinical diagnostic and management tools will be discussed.

C36. Pattern of *p63* mutations and their phenotypes in human ectodermal dysplasia syndromes

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Heterozygous mutations in the transcription factor gene *p63* are causative at least for six syndromes with various combinations of ectodermal dysplasia, orofacial clefting and limb malformations. Symptoms in these syndromes are overlapping, but different enough to be considered as a single syndrome. EEC and AEC syndromes present a strong genotype-phenotype association, whereas association in the other syndromes is more ambiguous. We present the results of an extensive study, which is based on 227 patients carrying a *p63* mutation. This study confirms previously observed genotype-phenotype associations. Yet, we also demonstrate that a remarkable phenotypic variation can be recognized in individual syndromes, even among patients carrying the same *p63* mutation.
Almost 90% of the EEC syndrome mutations are caused by five mutations. The phenotypic delineation of these "hot spot" mutations reveals that each of these five individual mutations imposes different risks towards specific aspects of the complete phenotypic spectrum. For example, patients with a R227 mutation rarely have facial clefts, whereas these are seen in >80% of patients with a R304 mutation. Likewise, kidney and urethral problems were observed in 40% of patients with an R227 mutation, but never among patients with a R280 mutation. These and other mutation-specific characteristics may reflect the importance of these amino acids in a certain developmental event. Furthermore, ADULT syndrome presents a "hot spot" mutation R298, and clarifies the phenotypic difference between Limb-Mammary syndrome. Finally, this study illustrates the minor differences between AEC and RHS, which let us to consider them to be a single entity.

C37. Haploinsufficiency of the *Euchromatin Histone Methyl Transferase1* (*Eu-HMTase1*) gene causes the 9q subtelomeric deletion syndrome.

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A rapidly growing number of patients with submicroscopic telomeric deletions of chromosome 9q has defined the definition of the clinically recognisable 9q subtelomeric deletion syndrome (9q- syndrome). Common features in these patients are severe mental retardation, hypotonia, brachycephaly, flat face with hypertelorism, synophrys, anteverted nares, thickened lower lip and carp mouth with macroglossia and conotruncal heart defects. The minimal critical region responsible for this 9q subtelomeric deletion syndrome has been estimated to be less than 1 Mb.
Recently, we have characterised the breakpoints of a *de novo* balanced translocation t(X;9)(p11.23;q34.3) in a female with features strikingly similar to those seen in the 9q$^-$ syndrome. The chromosome 9 breakpoint disrupted the *Eu-HMTase1* gene in the critical region at 9qter, which encodes Euchromatin Histone Methyl Transferase 1. Mouse tissue *ISH* of *Eu-HMTase1*, indicates that this gene plays an essential role in early embryonic development and that it is selectively expressed in adult brain. These observations suggest that disruption of *Eu-HMTase1* is causative for the 9q- syndrome. To proof this, we have developed an MLPA-based screening protocol for microdeletions in the *Eu-HMTase1* gene. MLPA analysis of a set of about 20 patients with a clinical presentation reminiscent of the 9q subtelomeric deletion syndrome revealed 4 additional microdeletions. Moreover, we identified a *de novo* nonsense mutation in the *Eu-HMTase1* gene in a clinically typical 9q- patient. In conclusion, these results establish that haploinsufficiency of *Eu-HMTase1* is causative for the 9q subtelomeric deletion syndrome.

C38. Duplications of the MECP2 region are commonly found in a specific subset of MR patients; towards a detailed genotype-phenotype correlation

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By full coverage X-array-CGH we identified a 0.4 Mb duplication at the MECP2 region in a family with severe mental retardation and progressive spasticity. Screening by real time quantitation (qPCR) of 17 additional MR patients with similar phenotypes revealed 3 additional duplications. Female carriers show skewed X-inactivation and MECP2 expression levels in PBLs of affected males demonstrated the double dosage. These findings revealed a yet unidentified mechanism for mental and developmental impairment (Van Esch et al., AJHG 2005). In order to assess the prevalence in the XLMR population we screened 124 randomly selected MR patients as well as 60 patients that were negative for MECP2, L1CAM or ATRX mutations by qPCR. Only one additional case from the ATRX negative group was identified. In collaboration with other clinical groups, another 11 carefully selected patients were found which harbor duplications of the MECP2 gene. By qPCR the location and extent of all 16 duplications were fine-mapped up to 5-10 kb resolution. The duplication sizes ranged from 0.3 to 1 Mb with the smallest overlapping region of 0.2 Mb only containing the IRAK1 and MECP2 genes. In some patients, the known MR genes SLC6A8, L1CAM or GDI1 were included. Interestingly, all proximal as well as distal breakpoints seem different which does not point to a common recombination mechanism. Based on this duplication mapping and the well characterized clinical features of the affected males, we performed a detailed genotype/phenotype correlation study of which the results will be discussed.

C39. Glyc-O-Genetics of Walker-Warburg Syndrome and related disorders

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O-linked glycosylation defects are the underlying cause for a group of disorders, which phenotypically are characterized by congenital muscular dystrophy (CMD) with mild to severe brain- and eye anomalies. Walker-Warburg syndrome (WWS) patients display the most severe developmental defects in this group of disorders, and generally die within the first year of live. A combined approach of homozygosity mapping in consanguineous families and candidate gene selection resulted in identification of mutations in *POMT1*, *POMT2*, *FCMD*, and *FRKP*. Mutations in these four genes are causative in nearly one third of all WWS patients in our cohort. Homozygosity data from 12 sporadic WWS cases and four families suggest involvement of multiple other loci. In order to identify the remaining WWS genes, we are screening genome-wide homozygosity data for candidate loci and use bioinformatics approaches focused on identification of candidate genes in these loci that functionally interact with known WWS genes. In addition, we aim to gain more insight in function and characteristics of known WWS genes, i.e. by studying genotype-phenotype correlations for genes involved in WWS and related disorders. Recently this resulted in the identification of *POMT1* mutations in patients with a less severe phenotype than WWS, characterized by CMD with calf hypertrophy, microcephaly, and mental retardation. We postulate that in these patients one or both transcripts for POMT1 confer residual protein O-mannosyltransferase activity. This suggests the existence of a disease spectrum of CMD including brain and eye abnormalities resulting from *POMT1* mutations.

C40. Transcriptome plasticity through RNA editing

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RNA editing by adenosine deamination, catalyzed by the adenosine deaminases acting on RNA (ADARs), is a posttranscriptional mechanism for the regulation of gene expression and particularly widespread in mammals (1). A-to-I RNA editing regulates important functional properties of neurotransmitter receptor genes in the central nervous system by changing single codons in pre-mRNA. The deficiency or misregulation of editing has been implicated in the etiology of neurological diseases, such as epilepsy, amyotrophic lateral sclerosis (ALS), depression and brain tumor progression.
We have recently identified Alu repeat elements in the human genome as a major target for post-transcriptional processing by A-to-I RNA editing (2). These findings suggest additional roles for RNA editing and links it to other RNA processing phenomena, such as alternative pre-mRNA splicing as well as siRNA mediated gene silencing and miRNA function (3).
In a new bioinformatics screen we have identified additional candidate genes for editing and experimentally confirmed cases where editing alters codons and leads to amino acid changes in the affected proteins.
The goal of our current work is to become able to predict which genes are subject to RNA editing, what additional functions it serves in vivo and how changes in RNA editing contribute to human diseases.
1. Maas et al., 2003, JBC 278, 1391-1394
2. Athanasiadis et al., 2004, PLoS Biology, 2 (12), e391, 1-15
3. Yang et al., Nat Struct Mol Biol. 2006 13, 13-21

C41. Life without sulfatases: exploiting a mouse model of multiple sulfatase deficiency

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Sulfatases are involved in several biological functions as diverse as degradation of complex molecules, production of steroid hormones and cell signaling. Humans have 17 different sulfatases. Eight diseases due to sulfatase deficiencies are known, while little is known for the nine remaining sulfatases. In Multiple Sulfatase Deficiency (MSD), the activities of all sulfatases are substantially reduced due to a defect in their post-translational modification. We have identified the *Sulfatase Modifying Factor 1 (SUMF1)* gene which is involved in the post-translational modification of sulfatases and is mutated in patients with MSD. We have now generated a *Sumf1* KO mouse model. Preliminary analysis of homozygous *Sumf1* KO mice revealed neonatal lethality, severe growth deficiency and massive accumulation of glycosaminoglycans (GAGs) in all examined tissues. The activities of five sulfatases tested were found completely absent. These data indicate that sulfatase post-translational modification in mammals absolutely requires *SUMF1*, thus excluding alternative mechanisms and suggesting that the residual sulfatase activities observed in the patients are the result of hypomorphic *SUMF1* mutations. Interestingly, heterozygous animals had consistently lower levels of all sulfatase activities compared to their wild type littermates reinforcing our previous hypothesis of *SUMF1* being not only an essential but also a limiting factor for sulfatase activity.
In this exceptional mouse model, the function of an entire protein family has been completely abolished, thus offering an unprecedented opportunity to study disease mechanisms and gene function in mammals.

C42. Altered activity of AP-1 transcription factor components in cystic kidneys of humans and mouse models for Autosomal Dominant Polycystic Kidney Disease.

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common inherited disorder that predominantly manifests with the formation of fluid-filled cysts in both kidneys. The disease is caused by a mutation in the *PKD1* or *PKD2* gene. To get insight in the crucial initial pathogenic events leading to cystogenesis we have created several mouse lines in which a large part of *Pkd1* is either deleted, or flanked by loxP sites. We also generated mice with a hypomorphic allele with reduced *Pkd1* gene expression, which are viable, showing bilaterally enlarged polycystic kidneys. Polycystin-1, the *PKD1* gene product, has been implicated in several signaling complexes that are known to regulate essential cellular functions. We demonstrated previously that aberrant expression of polycystin-1 results in modification of activator protein-1 (AP-1) transcription factor activity in cultured renal epithelial cells. Also in cystic kidneys of ADPKD patients and of homozygous hypomorphic *Pkd1* mice, AP-1 activity is altered. Interestingly, small cysts and/or dilated collecting ducts, representing initial stages of cyst formation, displayed highest expression levels of activated forms of ATF2, c-Jun, and of c-Fos.
Recently, we generated mice with conditional *Pkd1* deletion in renal epithelial cells, using a tamoxifen-inducible, kidney-specific *Cre* transgene. Biallelic inactivation of *Pkd1* in adult mice resulted in a minor cystic phenotype in the kidneys. However, inactivation of *Pkd1* in 4-days-old newborn mice resulted in rapid cyst growth. Again, significant up-regulation of activated forms of ATF2 and c-Jun was observed. Our data suggest that altered AP-1 activity might play an important role in early cystogenesis.

C43. The mutation in the Renin Receptor (ATP6A2) associated with XMRE (X-linked MR-epilepsy) significantly reduces ERK 1/2 activation by NGF in neurites

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A neutral mutation (p.D107D) in the renin receptor (ATP6A2) in a family with X-linked mental retardation and epilepsy (XMRE) resulted in exclusion of exon 4 (RERΔ4), as it altered an exon splice enhancer site (Ramser et al. HMG, 2005).
In order to further characterize its function and potential role in brain development, we have transfected both wild-type and mutant RER cDNA into rat PC-12 pheochromocytoma cells. The RERwt was particularly localizated to an area near the nucleus and to the tips of neural projections. However, RERΔ4 did not appear to accumulate at neurite tips. No other morphological differences between PC-12 cells expressing RERwt and RERΔ4 were evident. Unstimulated PC-12 cells expressing RERwt exhibited 2-3 fold increased levels of ERK1/2 phosphorylation compared to controls (empty vector transfected PC-12 cells), whereas their RERΔ4 counterparts demonstrated 1/6 the level of ERK1/2 phosphorylation relative to controls. Moreover, nerve growth factor stimulated RERΔ4 transfected cells exhibited 1/3 the level of ERK1/2 activation compared to controls and wild-type transfected cells. Thus, RERΔ4 appears to act in a “dominant-negative” manner to inhibit NGF-mediated ERK1/2 phosphorylation. Using differentially-epitope-tagged RERwt and RERΔ4, we determined that RERΔ4 can dimerize with itself (RERΔ4- RERΔ4), as well as with wild type RER (RERΔ4- RERwt). Together, our data suggest that the RERΔ4 mutation associated with XMRE may result in a dominant-negative form of renin receptor and may impair the ability of neurites to respond to NGF via the ERK1/2 pathway. These findings confirm the important role of the RER in cognitive functions.

C44. Mutant Connexin 26 Enhances Epidermal Wound Healing And Inhibits Bacterial Invasion

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A large proportion of recessive non-syndromic hearing loss is due to mutations in the GJB2 gene encoding connexin 26 (Cx26), a component of the gap junction. Within different ethnic groups, there are specific common recessive mutations each with a carrier frequency of between 1-3 % suggesting a possibility of heterozygous advantage. Indeed, carriers of the R143W-GJB2 allele, the most prevalent in the African population, present with a thicker epidermis than non-carriers and in an *in vitro* study we have previously demonstrated that human keratinocytes expressing R143W-Cx26 mutant are protected from cell death compared to wild-type Cx26. In our study, we investigated the role of wild-type Cx26 and R143W-Cx26 in epidermal wound healing by focusing on key features of a regenerating epidermis. We show increased migration and proliferation in cells expressing R143W-Cx26 compared to wild-type Cx26 counterpart. In addition, R143W-Cx26 expressing keratinocytes form a significantly thicker epidermis in an organotypic co-culture skin model. We also demonstrate that cells expressing this mutant Cx26 are significantly less susceptible to cellular invasion by the enteric pathogen *Shigella flexneri*. These studies demonstrate the advantageous effect of R143W-Cx26 in epithelia by enhancement of cellular aspects of epithelial wound healing and an inhibitory effect on bacterial invasion. These data suggest that loss of Cx26 expression either through GJB2 mutation or topical inhibition may have beneficial effects on the rate of wound repair and protection from infection.

C45. Gene expression profiling reveals a new molecular pathway involved in Oculopharyngeal Muscular Dystrophy

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Oculopharyngeal muscular dystrophy (OPMD) is a late-onset, usually autosomal dominant myopathy. OPMD is caused by a small expansion of an alanine (Ala) tract at the N-terminus of the nuclear poly(A)-binding protein (PABPN1) and is clinically characterised by progressive ptosis, dysphagia and limb muscle weakness. The pathological hallmark is the accumulation of unique tubulofilamentous inclusions within the nuclei of skeletal muscle fibers. These inclusions contain PABPN1 and also sequester ubiquitin, proteasome subunits and heat shock proteins as well as poly(A) RNA.
To model OPMD in a cellular system, we generated stable cell lines of primary mouse myoblasts expressing either wild-type (WT) or 7 alanine-expanded (+7Ala) PABPN1 under the control of the muscle-specific human desmin control region.
Following fusion into myotubes, these cells form intranuclear inclusions, which stain for PABPN1, ubiquitin, poly(A)polymerase (PAP) and poly(A) RNA, similar to the pathological hallmarks seen in OPMD patients. Large-scale gene expression analysis reveals a group of genes which is differentially upregulated in a trend comparable to PABPN1. A separate group of genes showes a significant downregulation upon PABPN1 overexpression, and the effect is strongly correlated with the percentage of inclusions present in the cells. The genes in this group are largely extracellular matrix (ECM) components or involved in the synthesis of the ECM. This is the first indication for involvement of the ECM in OPMD, which should lead to a better understanding of the disease mechanism and possible therapeutic intervention.

C46. Non invasive screening and rapid QF-PCR assay could reduce the need of conventional cytogenetic analyses in prenatal diagnosis

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Recently it has been shown that prenatal diagnosis by QF-PCR can detect the great majority of chromosome abnormalities, 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.
We developed a QF-PCR assay that was applied on 28.000 clinical samples with results issued in 24 hours. Common referral indications were: biochemical risk (32%), maternal age (30%), parental anxiety (22%) abnormal ultrasound (7 %) increased nuchal translucency (6%). All samples were also tested by cytogenetic analysis.
QF-PCR was normal in 26755 cases without false positive results. All

1030 aneuploidies involving chromosomes 21, 18, 13, X and Y were identified with 100% specificity. Several cases of partial trisomies and mosaicism were also detected.
QF-PCR showed 100% sensitivity in identifying clinically relevant abnormalities in samples referred for advanced maternal age and biochemical risk. In fetuses with abnormal ultrasound sensitivity was 95%. QF-PCR proved highly reliable allowing termination of affected pregnancies without waiting for cytogenetic analysis.
Our results raise the possibility of reducing the load of prenatal cytogenetics if pregnancies are monitored by non invasive tests. Women with positive results are offered amniocentesis or CVS and QF-PCR should always be performed. In case of abnormal QF-PCR results cytogenetic analyses may not be necessary as, in our experience, parents have opted for early termination in most cases of autosomal trisomies. In cases of negative QF-PCR results cytogenetic analyses might only be performed for fetuses with abnormal ultrasound

C47. Chromosomal mosaicism, DNA methylation and embryolethality: a possible link between cytogenetic and epigenetic factors in the etiology of embryo aneuploidy

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Progress in preimplantation genetic diagnosis as well as introduction of molecular cytogenetic techniques into analysis of human reproductive wastages have revealed a high frequency of chromosomal mosaicism. The presence of cell lines with different karyotypes is the consequence of mitotic errors during early stages of embryo development. However the molecular mechanisms of mitotic non-disjunction in embryo cells remain obscure. One of the most significant factors of genomic instability is inactivation of the cell cycle checkpoints. Several studies have proved that aberrant promoter DNA methylation is a strong mechanism for gene silencing. A global epigenetic genome reprogramming takes place during early stage of mammal's development. It is possible that there is a close relation between disturbances of the genome reprogramming and mitotic instability in the etiology of embryo aneuploidy. To test this hypothesis a pilot analysis of DNA methylation in promoters of the p16/pRB-pathway genes in 11 spontaneous abortions with chromosomal mosaicism was carried out. *CDKN2A* and *RB1* hypermethylation was found in the cytotrophoblast of 82% and 56% spontaneous abortions, respectively. The corresponding values in the extraembryonic mesoderm were 91% and 88%. None of the mosaic embryos without hypermethylation of these genes were found. At the same time no cases of aberrant methylation were found in the placental tissues of pregnancy term-related 23 induced abortions. Our preliminary results point out a possible link between aberrant epigenetic processes and etiology of mitotic non-disjunction in human embryos.
This work is supported by Grant of President of Russian Federation, MK-1969.2005.4.

C48. Ten years of a programme for presymptomatic testing (PST) and prenatal diagnosis (PND) in late-onset neurological diseases in Portugal: Machado-Joseph disease (MJD), Huntington disease (HD) and familial amyloid neuropathy type I - ATTRV30M (FAP-I)

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A programme for PST of MJD of late-onset neurological diseases is in place in Portugal since late 1995. A multidisciplinary team was gathered in each national centre for genetic counselling and psychosocial evaluation, and follow-up. Periodic workshops take place to develop and discuss intervention and research protocols, and compare experiences. IBMC is also the national reference lab for genetic testing of these disorders.
At CGPP-IBMC, in Porto, we received a total of 998 requests for PST or PND of late-onset neurological disorders: FAP-I (728), HD (142), MJD (67), FRDA (48) and other (23 for CADASIL, Wilson or SCA2), including 83 requests for PND.
Age of consultands ranged 17-80 years. Most had a partner (50.3%), 45.4% were single, 2.8% divorced, and 1.5% widows. Women (59%) predominated. Nearly 61% already had some children; only 57.5% were still in their reproductive years. About 85% reached delivery of results; 51% tested 'non-carriers', 49% 'carriers'.
The majority (48%) of our consultands came for PST mainly because of their "wish to know"; 15% mainly worried about children they had; 13% mainly for family planning and their "wish to have children"; 6% for "general life decisions"; 4% claiming "easier access to a treatment"; 4% "pressed by others"; and 7% (with a pregnancy ongoing) came with a simultaneous request for PND and PST. This reinforces the view that family planning, and the wish not to pass the mutant gene on to their children still to be born, are not the most important motivation for requesting and uptaking PST.

C49. Single-cell chromosomal imbalances detection by array CGH

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Single-cell genetic analysis has important applications for basic research as well as for clinical purposes. Thus far, genome wide analysis of the DNA of a single cell has remained cumbersome. Array Comparative Genomic Hybridization (CGH) is a new method that can detect chromosomal copy number changes across the genome. Thus far, array CGH of isolated single cells has been challenging due to the low amount of DNA present in a single cell. By optimizing a non-specific Phi29 DNA polymerase amplification of all genomic sequences, we generated sufficient quantities DNA of adequate quality for array CGH. Amplified DNA from a single cell of respectively a trisomy 13, 18, 21, and monosomy X cell line was hybridized on a 1-Mb array versus normal control DNA. All aneuploidies were unambiguously identified and, repeated experiments, showed the reproducible and accurate detection of the chromosomal aneuploidies being studied. In addition to aneuploidy detection, we demonstrate that also segmental aneusomies can be accurately identified. A fast protocol was generated allowing data interpretation in two days. This makes the method applicable for preimplantation genetic diagnosis. Currently, we are performing aneuploidy screening on single human blastomeres and results of these experiments will be presented.

C50. Whole genome amplification with haplotype analysis - a novel approach to preimplantation genetic diagnosis (PGD) for a wide range of diseases.

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PGD for single gene disorders is generally restricted to diseases with a common mutation, such as the DF508 cystic fibrosis mutation, and the number of diseases offered is limited by lengthy optimisation of assays at the single cell level. We present here a novel approach that overcomes these limitations by using a generic step of whole genome amplification (WGA) of single embryonic cells, followed by the disease-specific component of the test to obtain extended haplotypes spanning the disease locus. WGA of single cells is subject to allele drop-out, but this does not compromise diagnosis when using extensive marker panels.
The proof of this principle was demonstrated by successfully obtaining Dystrophin haplotypes on 9 single buccal cells from a female donor. Thus two Dystrophin multiplex assays containing 12 intragenic STR polymorphisms and 3 sex specific loci can be used to identify healthy males, non-carrier females, female carriers and males affected with Duchenne/Becker muscular dystrophy.
WGA and PCR analysis was carried out on 49 single blastomeres collected from 8 human embryos (including 3 sibling pairs) using a total of 57 polymorphic markers for chromosomes 1, 7, 13, 18, 21, X and Y. Results were obtained for each blastomere, demonstrating that

this approach can be applied without technical difficulty.
Linkage assays have been set up for cystic fibrosis, junctional epidermolysis bullosa, and recurrent Prader-Willi syndrome. The combined techniques of WGA and haplotyping thus open the way to the rapid, straightforward development and application of PGD tests for any mapped single gene disorder.

C51. Preimplantation genetic diagnosis for HLA compatible and disease free embryos: Single center experience.

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Stem cell transplantation from 'saviour' siblings have been a valuable alternative for therapy of a variety of monogenic disorders, hematologic malignancies and myelodysplasias. Between 2002 and 2005, 67 referrals to our center have been evaluated for eligibility to PGD of HLA match and monogenic disorder. After stringent selection criteria, 56 couples have been found eligible for PGD. The others have been rejected for reasons including unreproductive age, medical condition of the affected child, unproper expectations of the family, economic reasons, but most frequently decline of transplantation indication by the hematologists. PGD is performed on one or two single blastomeres biopsied from 6-8-cell embryos on day 3 after fertilization of the egg. HLA-A, HLA-B and HLA-DR regions and disease (beta thalassemia, Fanconi C, Fanconi A, SCID or Wiskott Aldrich syndrome) associated gene were initially amplified by multiplex PCR after the blastomer lysis. The embryos selected for HLA alleles by alternative methods including sequence based typing, real time PCR or STR haplotyping. Mutations of monogenic disorders have been tested by sequencing or by real time PCR. Total of 8 pregnancies have been obtained which has resulted already 4 deliveries. One cord blood has been transplanted to 12 year old sibling with beta thalassemia. We have experienced that PGD for HLA match and monogenic disease is feasible and provides valuable source for transplantation.

C52. Evidence of in vivo increase of SMN RNA and protein in SMA carriers and patients treated with valproic acid

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Proximal spinal muscular atrophy (SMA), an autosomal recessively inherited motoneuron disorder, leads to death in childhood in about half of all patients. While the disease is caused by homozygous absence of the survival motor neuron gene 1 (SMN1), each patient retains 1-4 SMN2 copies. They are almost identical to SMN1, but exon 7 is skipped in 90% of SMN2 transcripts. The encoded protein is not fully functional. Using fibroblasts from SMA patients, we have previously demonstrated that therapeutic doses of valproic acid (VPA), an antiepileptic drug, increase full-length (FL) SMN2 mRNA/protein levels in-vitro by enhancing SMN2 transcription and promoting exon 7 inclusion. These findings opened an exciting perspective for a causal SMA therapy.
Here, we provide a first proof of principle of an in-vivo activation of a causative gene by VPA, a histone deacetylase (HDAC) inhibitor, in a human inherited disease. Ten SMA carriers with 1 SMN1 and 1-3 SMN2 copies were enrolled in a VPA pilot trial. Drug treatment revealed increased FL-SMN mRNA/protein levels in blood from 7/10 probands. In a subsequent investigation of peripheral whole blood from 20 SMA type I-III patients treated with VPA in individual experimental curative approaches, FL-SMN2 mRNA levels were found to be increased in 7 patients, whereas 13 presented unchanged or decreased transcript levels. Difficulties in developing effective clinical biomarkers are pointed out and a strategy is suggested how to monitor drug response in treated patients. This will be an essential tool required for discriminating between responders and non-responders to VPA treatment.

C53. Valproic acid stimulates ABCD2 gene expression: a novel potential therapy for X-adrenoleukodystrophy

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Our ultimate goal is to develop new therapies for X-linked adrenoleukodystrophy (X-ALD), the most frequent inherited monogenic demyelinating disease (minimal incidence 1:17,000). X-ALD leads to death in boys due to cerebral demyelination (cerebral childhood ALD, CCALD) or to motor disability in adults due to spinal cord and peripheral nerve degeneration (adrenomyeloneuropathy or AMN). The gene mutated in the disease (ABCD1) is a peroxisomal ATP-binding transporter of very-long-chain-fatty acids, whose accumulation is the hallmark of the disease. We have generated and characterized mouse models for X-ALD by inactivation of ABCD1 and of a close homolog, the ABCD2 peroxisomal transporter. Recently, we have shown that stable overexpression of ABCD2 is able to prevent the late-onset neurodegenerative phenotype presented by ABCD1 knock-out mice. This constitutes an *in vivo* evidence of the overlapping functions of both transporters in the mouse. Because ABCD2 is a target of histone deacethylator (HDAC) inhibitors such as 4-phenylbutyrate, we investigated the effect of valproic acid (VPA), an HDAC inhibitor successfully used for the long-term treatment of epilepsy. Indeed, VPA stimulates ABCD2 expression *in vitro* and *ex vivo* in mouse and human. When given to X-ALD patients, a 2 to 4 fold upregulation of ABCD2 in peripheral mononuclear cells in 50% of the patients is reached. Thus, our findings open encouraging perspectives for the therapy of this devastating disease.

C54. Pharmacologic Chaperone AT2101 Improves the Trafficking and Activity of Acid-ß-Glucosidase in Gaucher Fibroblasts

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Gaucher disease is caused by mutations in acid β-glucosidase (GBA), the lysosomal enzyme responsible for the catabolism of glucosylceramide. The most common homozygous mutation in the GBA enzyme (N370S) has been shown to result in reduced activity and delayed trafficking of GBA to the lysosome in patient fibroblasts. Defective GBA activity results in the progressive accumulation of glucosylceramide within macrophages and osteoclasts, and GBA retention in the ER with increased protein degradation and activation of cytokines and release of inflammatory mediators ultimately leading to a variety of clinical manifestations such as anemia, bone fragility and possible CNS impairment. Currently, enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) represent the only viable treatment options for patients with Gaucher disease. More recently, the use of pharmacological chaperones as a means of rescuing GBA activity in Gaucher fibroblasts has been explored. In this study, we demonstrate that treatment of N370S fibroblasts with the glucose analog AT2101 (30-100 µM) increases the lysosomal steady-state levels of the GBA protein to near wild-type levels with a corresponding two-fold enhancement in GBA activity. Pulse/chase experiments indicate that AT2101 acts to both increase the intracellular trafficking of newly synthesized GBA and prolongs the stability of the mutant enzyme within the lysosome. Although a glucose analog, AT2101 is a very poor inhibitor of alpha-glucosidase II and does not alter N-linked oligosaccharide processing at 500µM. Taken together, these data suggest that AT2101 may be a promising alternative to enzyme replacement therapy (ERT) for the treatment of Gaucher disease.

C55. Intrabody-based therapy for protein aggregation disorders: OPMD as paradigm

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Oculopharyngeal muscular dystrophy (OPMD) is regarded a paradigm

for the group of protein aggregation disorders. It is caused by extensions of the N-terminal polyalanine stretch of the nuclear polyA-binding protein 1 (PABPN1) causing the presence of PABPN1-containing intranuclear aggregates in skeletal muscle. Intranuclear aggregation of mutant PABPN1 is also observed in transgenic mouse and cell models for OPMD and these models consistently support a direct role for the protein aggregation in OPMD pathogenesis. We have isolated and characterized several single-domain antibody reagents (VHH) that recognize at least two different epitopes in PABPN1. The antibody reagents specifically detect endogenous PABPN1 in cell lysates on western blot and label PABPN1 in cultured cells and muscle sections. When expressed intracellularly as intrabodies in a cellular model for OPMD, aggregation of PABPN1 was prevented in a dose-dependent manner. These intrabodies have also curative properties as they could also reduce the presence of already existing aggregates. Given the domain specificity of VHH-mediated aggregation interference, this approach allows the definition of the nucleation kernel in aggregation-prone proteins, thus facilitating etiological insight into this and other protein aggregation disorders. It may also provide useful therapeutic agents.

C56. Advances in exon skipping trial development towards proof-of-concept and systemic application in Duchenne Muscular Dystrophy

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Antisense oligonucleotides (AONs) are emerging as small molecule drugs with a high, sequence-specific, corrective potential on RNA level. Especially for Duchenne muscular dystrophy (DMD), AONs have demonstrated to be an efficient and relatively simple and safe alternative to gene therapy approaches based on gene replacement. Over the last five years we have successfully developed this antisense approach towards clinical application. In the first half of 2006, a “proof-of-concept” clinical study, based on intramuscular injections of an exon 51 skipping AON, will be undertaken in a selected group of DMD patients, of which the set-up will be discussed. To facilitate full-body treatment of DMD patients, we are now focusing on the development of a safe and efficient systemic AON-delivery method. Using the *mdx* mouse model, we have compared efficacy, persistence and biodistribution of an AON (containing 2‘-O-methyl RNA with a full-length phosphorothioate backbone) targeting the mutated exon 23, after intravenous administration of increasing doses and intervals. We obtained relatively high exon 23 skipping levels (up to 45%) in all muscle types, including diaphragm and heart, resulting in significant dystrophin expression as detected by Western blot analysis. There was an increased muscle-specific uptake and efficacy of AON in the *mdx* mice when compared to control mice. A highly sensitive ligation ELISA for AONs, allowed us to assess the stability of the AON in the different organs and tissues, showing a half life of 10 days in skeletal muscle. The overall therapeutic effect was indicated by significantly decreased creatin kinase levels in plasma of treated mice, and by improved performance in RotaRod studies. These results offer a favourable perspective for the first clinical Phase I/IIa studies on AONs in DMD patients, suggesting that these are within reach in the next few years.

C57. Clinical burden and penetrance of haemochromatosis: estimates derived from routine data

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The prevalence of the at-risk genotype for haemochromatosis is common at approximately 1 in 200, the clinical penetrance is unknown. This study aims to determine the clinical burden of haemochromatosis and provide an estimate of the probability of diagnosis and treatment for C282Y homozygotes in a Northern European population. The prevalence and incidence of haemochromatosis requiring hospitalisation together with associated co-morbidity, patterns of care and lifetime cumulative probability of admission was estimated from routine hospital admissions data for England. The data covered six years from April 1997 to March 2003 and consisted of 56000 episodes of care relating to 6000 individual patients with a diagnosis of haemochromatosis.
Crude prevalence was 53 per million of population (pmp) and crude incidence 21 pmp in 2002/3. Indirectly age sex standardized prevalence by health authority of residence indicated unexplained geographical variation. Lifetime probability of having an episode of care related to haemochromatosis was 9% in males and 4% in females in a hypothetical cohort of 1000 C282Y homozygotes. 14% of patients had liver disease recorded at the first episode of care. The majority of care was provided by haematologists.
Despite a high prevalence of the at-risk genotype, haemochromatosis is uncommon. Lifetime penetrance was nearly 10% in males. A significant percentage of individuals present with preventable complications at their first episode of care. This together with geographical variation in prevalence suggests further scope for earlier diagnosis.

C58. Preference for sweet foods is partially genetically determined; a Finnish family study

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The human has an innate preference for sweet foods. However, the degree of sweet taste preference varies largely between individuals. The aim was to study if genetic elements contribute to these differences. The study population consists of 146 members (32 % male, 68 % female, 19 to 78 years old) of 26 Finnish families, genome-scanned with 360 microsatellite markers. The subjects rated the sweetness intensity and pleasantness of 0, 3, 7.5, and 18.75 % sucrose solutions using labeled magnitude scales LMS (Green et al. 1993) and LAM (Schutz & Cardello, 2001), and evaluated the pleasantness and use frequency of 5 sweet foods (chocolate, candies, ice cream, sweet pastry, sweet desserts) using 7-point scales. Phenotypes for pleasantness and use frequency of sweet foods were constructed as mean of ratings given to these 5 items. Program MERLIN was used for variance component linkage analysis of these quantitative traits. Highest heritabilities were observed for pleasantness of 7.5 and 18.75 % sucrose solution (h^2 = 23 and 48 %, respectively) and for sweet foods' pleasantness (h^2 = 19 %) and use frequency (h^2 = 35 %). The multipoint linkage analysis provided a LOD score of 3.49 at marker GGAA3G05 located in Chr16p11.2 to use frequency of sweet foods. These results suggest that the preference for sweet taste is partially genetically determined and that locus 16p11.2 harbors a genetic element modifying the use of sweet foods.

C59. A common genetic variant 10 kb upstream of *INSIG2* is associated with adult and childhood obesity

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Obesity is a heritable trait and a risk factor for many of the common

diseases such as type 2 diabetes, heart disease, and hypertension. Here we use a dense, whole-genome scan of the DNA samples from the Framingham Heart Study cohort to identify a common genetic variant near the INSIG2 gene (insulin-induced gene) associated with obesity. We have replicated the finding in four separate samples comprised of individuals of Western European ancestry, African Americans and children. One of the replication samples was the large population based KORAS4 study from Germany (n=3996). The obesity-predisposing genotype is present in 10% of individuals. Our study suggests that common genetic polymorphisms are important determinants of obesity.

C60. Genetic variants of RANTES are associated with protection for Type 1 Diabetes

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Type 1 diabetes (T1D) is an autoimmune disease with complex genetic predisposition. RANTES (CCL5) is a Th1 chemokine, which promote T-cell activation and proliferation. Genetic association of *RANTES* with asthma, sarcoidosis and multiple sclerosis have previously been described. Increased protein levels of RANTES at the primary sites of inflammation have been reported for different autoimmune diseases.

In this study we tested *RANTES* as a candidate gene for association with T1D, using three tagged SNPs to capture all haplotype information. The minor allele of all three SNPs was found to be less transmitted to T1D offspring (transmission rates 37.3%(p=0.002), 38.7%(p=0.007) and 41.0%(p=0.013)), and also less frequent in case versus control design (p= 0.009, 0.03 and 0.04 respectively). A similar protective effect was observed for the haplotype carrying all three minor alleles (TDT: p=0.003; OR=0.55; CI: 0.37-0.83, case/control: p=0.03; OR=0.74; CI: 0.55-0.98). Finally, individuals carrying the protective haplotype express significantly lower serum level of RANTES, when compared to non-carriers.

Conclusion: Genetic variation of *RANTES* is significantly associated with T1D, both in a case control and in family-based analysis. Carriers of the protective haplotype express lower serum level of RANTES protein, suggesting lower T-cell activation and proliferation.

C61. Screening and replication using the same data set: A testing strategy for case/control studies

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We propose a novel testing strategy for case/control studies that uses the same data set for the screening step and the replication step. For family-based designs, we proposed such 2-stage strategy in which both steps are statistical independent: the screening step, in which the strength of the genetic association between each marker and the trait is assessed, and the replication step, in which a selected subset of markers is tested for genetic association with the trait. However, the approach has been limited to family data. Here, we propose a testing strategy for case/control studies that uses the same data set for screening and replication, and makes the necessity of family-data for such testing strategies redundant. We outline the approach and assess its power compared to standard multiple-testing procedures. Its practical relevance for genome-wide association studies is illustrated by applications to genome-wide association studies.

C62. Will systematic association studies of anonymous SNPs succeed where linkage studies failed?

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Systematic linkage screenings for complex disease genes have often had poor results, despite the impressive number of diseases studied. As linkage studies have low power to detect common variants, it is hypothesized that common polymorphisms may be involved in complex disease. In order to detect the contribution of these alleles, large-scale association testing has been proposed as an alternative to linkage studies. Using maps of frequent SNPs, the entire genome, or more restricted regions of it, can be queried for association with a disease. These large-scale association studies have become a reality with the recent availability of the HapMap database and are a promising tool for understanding the genetic component of complex diseases.

However, the question of the actual power of this new approach for identifying genetic risk factors for complex diseases is still open. Power studies published in support of the approach were based on the hypothesis of a single variant associated with the disease. Because true complex disease etiology may include cases of several variants in one gene or gene-gene interactions, we perform power computation under several of these more complex models using the ENCODE data that exhaustively describes the SNPs in ten 500kb-regions of the human genome. We show that under very realistic models, including the scenario of common variants, the approach of systematic anonymous SNP association tests may also fail.

C63. Quantitative mapping of loci influencing susceptibility to lentiviral infection.

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Susceptibility to lentiviral infection (including HIV) is a quantitative trait that is likely to have a strong genetic component and be influenced by genetic variability of the host.

To identify the genetic components underlying this trait we established an ex vivo approach to measure lentiviral susceptibility in lymphoblastoid cells (LBLs), based on a GFP reporter system. The assay outputs two phenotypes: fraction of infected cells, and expression of lentiviral genes.

We measured lentiviral susceptibility in 198 LBLs from 15 three-generation CEPH families to calculate heritability and perform quantitative linkage using previously generated genotypes.

Heritability calculations showed that both phenotypes have a strong genetic component with h2r of 0.53 and 0.43 respectively. Quantitative linkage analysis using variance components, lead to the identification of a suggestive locus on chromosome 8q for the 'fraction of infected cells' trait (LOD=2.89, p=2E-04). Because the phenotype is not normally distributed we performed 500 simulations to determine the empirical significance of the finding; the LOD-score obtained was genome-wide significant (95% significance threshold = 2.83). To further dissect the susceptibility locus, we measured the trait in LBLs of the 60 caucasian HapMap individuals. We selected all tag SNPs in a 3Mb region centered on the marker with the highest LOD-score and performed association analysis, which identified a single significant SNP (p=7.7E-05) after correction for multiple testing. This finding narrows down the candidate region to 5-10 genes. We thus identify a new locus for lentiviral susceptibility that could help improve our understanding on the mechanisms of HIV infection.

C64. Distinctive white matter abnormalities on MRI in patients with 6p deletion syndrome

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A girl with mental retardation, short stature and a distinct pattern of multiple congenital abnormalities, including white matter abnormalities on MRI, presented at our department in 1999. Despite intensive investigations, no diagnosis could be made. Recently, the parents presented with their third child. He showed a similar pattern of congenital and MRI abnormalities as his sister. MAPH analysis, followed by FISH and Array-CGH, revealed a cryptic unbalanced chromosomal translocation leading to a monosomy of 6pter and a trisomy 20qter in both siblings. Based on analogous MRI findings, a third, unrelated patient, was diagnosed and molecularly confirmed to have 6p deletion

syndrome by her neuroradiologist.
The recent literature delineates a pattern of congenital abnormalities and dysmorphic features that should alert the clinician to the possibility of 6p deletion syndrome and instigation of appropriate investigations (FISH or MAPH/MLPA of the 6p telomeric region). In addition to the already described features, we would like to add the finding that children with 6p deletion syndrome seem to have a marked, and possibly pathognomonic, pattern of white matter abnormalities on brain MRI.

C65. Array-CGH: A novel tool in genetic diagnosis of individuals with congenital heart defects

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Array-CGH is a novel diagnostic tool for the detection of submicroscopic chromosomal imbalances. Moreover, it offers an opportunity to identify novel genetic loci for specific genetic disorders. We report the results of array-CGH analysis in 60 patients with a congenital heart defect (CHD) of unknown cause, who had, in addition, either developmental delay/mental retardation and/or additional major malformation(s) and/or dysmorphism.
Array-CGH was performed using a home-made 1Mb array, with the 1Mb BAC/PAC set from the Sanger institute. All detected anomalies were confirmed and parents were investigated.
New abnormalities were detected in 11/60 patients (18%). Mosaicism for monosomy 7 was detected in a patient with suspected diagnosis of Fanconi syndrome but normal DEB and mitomycin tests. Among the 10 others, there were 4 interstitial deletions (maximal sizes between 2 and 14 Mb), 1 interstitial duplication (6 Mb), a paternally inherited duplication in chromosome 22q11.2, 1 terminal deletion 5q (6 Mb), 1 unbalanced translocation (9q/20q) and two more complex intrachromosomal rearrangements involving deletions, duplications and inversions.
With the exception of TBX1 (dup 22q11.2), NOTCH1 (del 9q34.3) and NKX2.5 (del 5q35.12), no genes for CHD are known in the identified regions. Using ENDEAVOUR, a recently developed bioinformatics tool to prioritize candidate genes in specified chromosomal regions, we identified several novel candidate genes for CHD. Array-CGH can detect cryptic submicroscopic imbalances in a large proportion of patients with a CHD and a “chromosomal” phenotype. Moreover, the identified chromosomal imbalances are a source of novel candidate genes for CHD.

C66. Molecular cytogenetic (re-)examinations of structural chromosome aberrations within the ECARUCA (European Cytogeneticists Association Register of Unbalanced Chromosome Aberrations) project reveals surprising results

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Within the ECARUCA project, molecular cytogenetic re-examinations were performed for European laboratories with the aim to better define breakpoints in various structural chromosome aberrations. As the main aim of the Register is to better know and understand the phenotype and course of rare chromosome aberrations, it was considered important to precisely define the aneuploid segments with respect to karyotype-phenotype correlation. For deletions and duplications, microsatellite marker analysis and FISH with BACs was the method of choice. For additional marker chromosomes, chromosome dissection and reverse paiting was also applied. In selected cases also MLPA and array CGH was applied. More than 100 patients were so far examined. The initial determination of breakpoints had to be revised in almost all cases. In some patients even the chromosome involved had not been correctly determined. Deletions in some cases concerned segments which were not even overlapping with the initially determined segment. Especially large was the discrepancy in instances of duplication-deletions. From the above-mentioned results we conclude that many patients in whom especially de novo duplications, interstitial deletions and additional marker chromosomes were determined, should be re-examinated with molecular cytogenetic methods. This will result in many revisions of initial karyotypes and will improve our knowledge about the impact of the aneuploid state of specific chromosomal segments on the phenotype down to the gene level.

C67. Targeted cloning of fragile sites - based on a previous tagging of fragile regions in a breast cancer cell line

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Common fragile sites are chromosomal loci which can be observed as breaks or gaps on metaphase chromosomes after culturing the cells under conditions inhibiting DNA replication or repair. They reflect site-specific genetic instability being involved in e.g. chromosome rearrangements, sister chromatid exchanges and gene amplification. During the last years progress has been made in understanding the molecular basis of common fragile sites and the mechanisms which account for their expression, however, the nature of common fragile sites is still not completely known.
Point of departure for a targeted cloning of several fragile sites was tagging of fragile site DNA by repair mediated integration of a marker gene into breaks. For this approach, the breast cancer cell line MDA-MB-436 was chosen as model, since it spontaneously expresses fragile sites with a high frequency and displays other non-random instable regions. A transfected marker gene integrated preferentially into fragile sites and non-random instable regions (44 respectively 41% of total integrations) and thus tagging the fragile regions. Using different cloning approaches flanking sequences of the marker gene were identified and the exact position of the respective corresponding fragile sites was determined. The co-localization of the derived sequences with the fragile site was confirmed by fluorescence *in situ* hybridization on metaphase chromosomes of lymphoblastoid cell lines treated with Aphidicolin to induce fragile site expression. The exact dimensions of the fragile sites were determined by FISH mapping analysis.

C68. Genome wide tiling path array CGH analysis in a diagnostic setting. A three-year experience

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In April 2003, genome wide array-based Comparative Genomic Hybridisation (array CGH) analysis was diagnostically implemented in our cytogenetic services in order to reach a resolution that extends far beyond routine cytogenetic analysis[1]. This was achieved by the mutual efforts of people from five different fields of expertise: clinical genetics, cytogenetics, DNA diagnostics, bioinformatics, and biostatistics.
One year after implementation, we switched from a first generation 1 Mb resolution BAC array to a second generation 100 kb resolution tiling path array encompassing over 32,000 BAC clones. After tackling several practical high hurdles, one of the main being the correct clinical interpretation of the analytical data and the subsequent classification of imbalances as variant, unique or inherited, the first final diagnostic array CGH outcomes (of a current total of ~500 array requests) were completed and reported to the requesting physicians early in 2005.
Various practical aspects that have resulted in our current diagnostic strategy are discussed. These include cytogenetic and subtelomere Multiplex Ligase-dependent Probe Amplification (MLPA) analysis prior to array CGH analysis, and subsequent validation of significant array CGH aberrations by region-specific MLPA and/or FISH. Other relevant diagnostic issues such as parental testing, family and follow-up studies, and ISCN-based nomenclature are also addressed.
Our current strategy enables us to routinely use this powerful, yet expensive tool to detect submicroscopic imbalances and thereby to unravel the causative defects in a growing number of patients with mental retardation with or without multiple congenital anomalies.
[1]De Vries et al., Am. J. Hum. Genet. 77:606-616 (2005).

C69. Subtelomeric imbalances in phenotypically normal individuals

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Submicroscopic telomeric imbalances are a major cause of developmental and mental disorders and are detected in 3 to 7% of patients with normal karyotypes. Screening for subtelomeric aberrations has become a routine aspect of the diagnostic work-up of patients with MCA/MR. Because of this strong correlation between subtelomeric imbalances and developmental disorders, subtelomeric screening is being implemented in prenatal diagnostic techniques.
In contrast to these findings, scattered reports in the literature describe phenotypically normal individuals carrying subtelomeric imbalances. Here, we report on the detection of subtelomeric chromosomal imbalances in phenotypically normal individuals at another six subtelomeres of respectively chromosomes 4p, 6q 10q, 11q, 17p, 17q, and 18q. In addition, array CGH was applied and uncovered imbalances sized between 0.4 Mb and 7 Mb. Surprisingly, this shows that not only small chromosomal deletions and duplications, but also large imbalances can be tolerated without obvious phenotypic anomalies. None of these subtelomeric imbalances are listed as common copy number variations of the human genome. Since most of them have been ascertained through the detection of the imbalance in a child with a developmental disorder, they are likely to be susceptibility factors that, dependent on the genetic or environmental background, can lead to phenotypic anomalies. These observations raise major ethical concerns for the introduction of subtelomeric and genome wide screening tools in prenatal genetic diagnosis

C70. Cranio-lenticulo-sutural dysplasia is caused by a SEC23A mutation disrupting ER-to-Golgi trafficking

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We described Cranio-lenticulo-sutural dysplasia (CLSD) as a novel autosomal recessive syndrome with late-closing fontanels, sutural cataracts, facial dysmorphisms, and skeletal defects mapped to chromosome 14q13-q21. Using a positional cloning approach we identified F382L missense mutation in SEC23A segregating with this syndrome. SEC23A is an essential component of the COPII-coated vesicles that transport secretory proteins from the endoplasmic reticulum (ER) to the Golgi complex. Electron microscopy and immunofluorescence (IF) documented gross dilatation of the ER in patient fibroblasts. The cells also exhibited cytoplasmic mislocalization of SEC31 and delayed secretion of COL1A1. Transfection of fibroblast cells with a mutant SEC23A expression vector produced cellular phenotypes similar to those observed in the mutant cells. These observations were corroborated by in vitro cell-free vesicle budding assays which demonstrated that the F832L mutation results in loss of SEC23A function and inefficient COPII complex formation. We propose that a secretory defect of a distinct set of cargo proteins required for normal morphogenesis accounts for CLSD.

C71. The transmembrane protein meckelin (*MKS3*) is mutated in Meckel-Gruber syndrome

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Meckel-Gruber syndrome (MKS) is a severe autosomal recessively-inherited disorder characterized by bilateral renal cystic dysplasia, developmental defects of the central nervous system (most commonly occipital encephalocele), hepatic ductal dysplasia and cysts, and polydactyly. MKS is genetically heterogeneous with three loci mapped: *MKS1*, 17q21-24; *MKS2*, 11q13 and *MKS3*. We have fine-mapped the *MKS3* locus to a 12.67 Mb candidate interval at chromosome 8q21.13-q22.1. We sequenced 22 positional candidate genes before we identified pathogenic mutations in *TMEM67* for five *MKS3*-linked consanguineous families with classic clinical features of MKS.
MKS3/*TMEM67* is a novel, evolutionarily-conserved gene that is expressed in foetal spinal cord, brain, liver and kidney, as expected for the phenotype, as well as more general low levels of expression. It encodes a novel, 995 amino acid, transmembrane protein that we have called meckelin. Meckelin has topological similarity to the G-protein-coupled and the Frizzled (FZ) families of receptors, and may have a role in primary ciliary and basal body function. Further mutation analysis has identified a number of frame-shift, splice-site and missense changes, all of which are in exons encoding the extracellular domain of meckelin.
We present initial genotype-phenotype correlations for MKS, and preliminary functional work that begins to elucidate the role of meckelin in normal human development.

C72. Germline mutations of proto-oncogenes in the RAS-RAF-ERK pathway cause Costello syndrome and cardio-facio-cutaneous (CFC) syndrome

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Costello syndrome is a rare, multiple congenital anomaly syndrome characterized by coarse face, mental retardation, cardiomyopathy and predisposition to tumors. The molecular basis of the disease has been unknown. Mutations in tyrosine phosphatase SHP-2 (*PTPN11*) have been identified in approximately 40% of patients with Noonan syndrome[1], which phenotypically overlaps with Costello syndrome. We hypothesized that the causative gene(s) for Costello syndrome and Noonan syndrome without *PTPN11* mutations is a functionally upstream or downstream molecule(s) of SHP-2 in the RAS-RAF-ERK pathway. We sequenced the entire coding regions of the four RAS genes (*KRAS*, *HRAS*, *NRAS* and *ERAS)* in genomic DNA from 13 individuals with Costello syndrome and 28 individuals with *PTPN11*-negative Noonan syndrome. We identified four heterozygous *de novo* mutations of *HRAS* (G12V, G12A, G12S and G13D) in 12 of 13 affected individuals, all of which have been previously reported as somatic and "oncogenic" mutations in various tumors. Fibroblasts established from patients were hypersensitive to growth factor stimulation as compared with control fibroblasts. Only a mutant allele was expressed in the ganglioneuroblastoma tissue surgically isolated from an individual with Costello syndrome despite the biallelic expression in her fibroblasts. Furthermore, we recently identified genes mutated in CFC syndrome. Our observations strongly suggest that dysregulation of the RAS-RAF-ERK pathway is a common molecular basis for the three related disorders, Noonan, Costello, and CFC syndrome.

1. Tartaglia, M. & Gelb, B.D. *Eur J Med Genet* **48**, 81-96 (2005).
2. Aoki, Y. et al. *Nat Genet* **37**, 1038-40 (2005).

C73. Mutations in different components of FGF-signalling in LADD syndrome

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Autosomal dominant lacrimo-auriculo-dento-digital (LADD) syndrome is a multiple congenital anomaly mainly characterized by lacrimal duct aplasia, malformed ears and deafness, small teeth, and digital anomalies. We genotyped three large LADD families using the Affymetrix GeneChip 10K Array and mapped the LADD1 locus on chromosome 10q26 to a 6.2 Mb critical region between markers *D10S1693* and *D10S1723*. We identified heterozygous missense mutations in the tyrosine-kinase domain of the fibroblast-growth-factor-receptor 2 (*FGFR2*) in all three LADD families and a *de novo FGFR2* mutation in a sporadic case. After exclusion of *FGFR2* in two additional LADD families we found a causative mutation in the tyrosine-kinase domain of *FGFR3* in one family, and a missense mutation in the gene encoding fibroblast-growth-factor 10 (*FGF10*), a known FGFR ligand, in the other family.

We conclude that LADD syndrome is a genetically heterogeneous disorder caused by heterozygous missense mutations in either *FGFR2*, *FGFR3*, or *FGF10*. Interestingly, all FGFR mutations so far identified in LADD are located within the tyrosine-kinase domains of FGFR2 and FGFR3 in loops that play a regulatory function in the control of tyrosine-kinase activity. Therefore, reduced functional activity of FGFR2/3 seems to be an attractive plausible mechanism underlying the molecular basis of LADD syndrome.

C74. Further evidence and functional proof of the pathogenic relevance of *TBX1* missense mutations

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Deletion 22q11.2 syndrome is the most frequent known microdeletion syndrome. It is associated with a highly variable phenotype including DiGeorge- and Shprintzen syndromes.From studies in several mouse models, haploinsufficiency of the T-box transcription factor *TBX1*, which is located within the common deletion interval, was suggested to cause the phenotype. Nevertheless, to date only 3 patients from Japan were described to have point mutations of *TBX1* in association with five of the major features of 22q11.2 deletion. We report the first Caucasian patient with a *TBX1* missense mutation within the T-box, associated with the typical facial gestalt, short stature and developmental delay. To prove the functional relevance of this mutation we tested our novel and the three published mutations in a transcriptional reporter assay. While the published truncating mutation showed remarkable reduction of TBX1 transcriptional activity, the published and our novel missense mutation showed significantly increased activity. While RNA levels showed no increase of transcript, homology modelling of the mutant protein showed new electrostatic interactions, which could enhance the stability of dimers. We were able to confirm this hypothesis by an EMSA assay using the T-box binding site. This unexpected results are in line with data from a mouse model, which showed a similar phenotype in both, *TBX1* under- and over expression. We therefore provide the first functional evidence for the pathogenic relevance of *TBX1* missense mutations.

C75. Branching and nucleokinesis defects in migrating interneurons derived from doublecortin knockout mice

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Doublecortin (DCX) is mutated in cases of lissencephaly, a malformation of cortical development associated with severe mental retardation and epilepsy. In this disorder the cerebral cortex is highly disorganised, most probably resulting from perturbed neuronal migration. We are pursuing a variety of approaches in an attempt to better understand the pathophysiology of lissencephaly. In addition to performing mutation screening analyses, we identified and characterised several protein partners of DCX, providing entry points into understanding the biochemical pathways in which it is implicated. These studies show that DCX is a MAP, also implicated in vesicle trafficking and cell adhesion, likely to be important functions for neuronal migration. We also generated *Dcx* knockout mice which interestingly, have only subtle abnormalities in the mouse cortex. Nevertheless, our video microscopy analyses of migrating knockout interneurons show defects in migratory dynamics. Specifically, the formation and division of growth cones at the extremities of neuronal processes are more frequent in knockout cells. As a consequence, cells are more extensively branched, although individual branches are less stable. Dcx-deficient cells thus migrate in a disorganised manner, extending and retracting short branches and making less long distant movements of the nucleus. These novel data thus highlight an important role for Dcx in migrating interneurons. Furthermore, comparative neuropathological analyses also identify severe defects in the distribution of interneurons in type I lissencephaly brains. Perturbations of the number, distribution or function of interneurons, which are important regulators of neuronal activity, are likely to contribute to the epilepsy observed in this disorder.

C76. Identification of DNA methylation markers for detection and classification of colon cancer by epigenetic profiling

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Epigenetic modification of gene expression by aberrant methylation of promoter CpG islands plays an important role in the initiation and development of colorectal cancer (CRC). DNA methylation-based markers are attractive because the aberrant methylation is thought to arise early in the development of CRC. In addition, the stability of methylation changes in the DNA allows the development of cost-effective and sensitive assays. Therefore, we aim at the identification of high-performance DNA methylation markers for the detection and classification of CRC and its precursor forms. We are carrying out genome-wide methylation studies in a consecutive series of clinically well-described colon tumors and paired normal samples using differential methylation hybridization of CpG island microarrays. Patient DNA samples derived from macrodissected fresh-frozen tissues are digested with MseI and amplified by linker-mediated PCR. Subsequently, the amplicons are digested by two methylation-sensitive restriction enzymes, Cy-labeled and co-hybridized with a common reference sample to microarrays containing 9,000 PCR-amplified CpG islands. We identified loci that are aberrantly methylated in a high proportion of tumor samples, including promising candidate biomarkers with methylation in over 90% of the tested tumors. Currently, these loci are being verified by bisulfite sequencing and studied in more detail. Possible clinical applications of these markers include early detection of cancer with applications such as in feces screening, and cancer prognosis.

C77. Identification of regulatory Conserved Non-Coding sequences (CNCs) using the chicken genome and chicken embryos

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Comparative genome analysis across mammals has revealed a large number of highly conserved non-coding sequences (CNCs). We had studied CNCs from 21q and found that their evolutionary features strongly suggested functional importance. However the function of the majority of these sequences is unknown. We hypothesised that a possible role of a subset of CNCs is transcriptional regulation.

In vitro luciferase and DNaseI hypersensitive assays were used to evaluate this putative regulatory function; the results of both screens revealed that only 15% of CNCs have transcriptional regulators properties. This supports the current hypothesis that there may be insufficient evolutionary distance between humans and others mammals to detect conserved regulatory sequences.

In that view, the chicken offers a more divergent genome for comparative analysis (310Mya for chicken-human common ancestor) and a tractable experimental system to evaluate the phylogenetic distance necessary for regulatory elements identification.

Alignment of 21q CNCs with the chicken genome revealed that ~10% of these are conserved down to birds. We electroporated chicken retinas (E3 & E6) and chicken whole embryos (E2) with GFP-reporter vectors to determine the regulatory potential of a set of those CNCs. Preliminary data on 11 sequences suggest that the majority of the tested CNCs (72%) strongly activate the GFP expression in explanted retina and in different embryonic tissues. We observed that few CNCs behave as enhancers in early retina (E3) but not at later developmental stages (E6).

Our results suggest that the chicken genome could be used to identify transcriptional regulators among the mammalian CNCs.

C78. Genome-wide copy number profiling on high density BAC, SNP and oligonucleotide microarrays: A platform comparison

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Microarray-based comparative genomic hybridization (array CGH) approaches have recently proven successful for detection of submicroscopic copy number alterations. Array CGH is now possible with a genomewide tiling resolution of ~100 kilobases using 32,000 BAC clones. Improvements in resolution can be achieved by using higher numbers of shorter fragments, provided that measurement precision is maintained. In this study we compared the performance of two commercially available oligonucleotide platforms to our tiling resolution BAC array platform. DNAs from ten patients with submicroscopic copy number abnormalities, identified by tiling resolution array CGH, were hybridized in a blinded fashion onto 100k Affymetrix SNP arrays and 385k Nimblegen oligonucleotide arrays. We implemented a recently published algorithm for SNP-based copy number detection, corrected for systematic variation using normal controls and automated data-analysis. Most known submicroscopic alterations were identified by both platforms, as well as many additional copy number variants smaller than one megabase. Statistical power analyses indicated that the BAC array platform exhibited the highest signal-to-noise ratio, allowing reliable detection of single copy number alterations encompassing only 4 BACs. More targets needed to be combined for reliable copy number detection on the Affymetrix and Nimblegen arrays. The higher target density of the latter two, however, compensated for the lower detection power and the smaller size of the targets allowed detection of alterations <100 kb. These findings indicate that genome profiling results can be comparable across platforms, and increases in target density will ultimately allow for copy number analysis of all 250,000 exons in the human genome.

C79. Detection of copy number changes in patients with mental retardation using high density SNP microarrays

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Array-based genomic screening is a powerful strategy to identify chromosomal and allelic rearrangements. Among these techniques, whole genome analysis using high-density SNP genotyping oligonucleotide arrays allow identification of yet unknown microdeletions, microduplications and uniparental disomies. We collected a cohort of 70 children and, when available, their parents with unexplained mental and developmental retardation, facial and/or sceletal dysmorphologies, and other symptoms as well. The first screening of the patients, including high resolution banding analysis and metabolic investigations was unconspicuous. The patients genomic DNAs were analysed using the Affymetrix GeneChip 100K Array, consisting of 116,204 single nucleotide polymorphism (SNP) probes with an average spacing of 23.6 kilobases. Data analysis was perfomed with dChip, Bioconductor and Perl script. Preliminary analysis revealed approximately 10% de novo rearrangements. These deletions and duplications varied in size from 200 kb to 10 Mb and span regions which contain up to 118 known genes. Rearrangements will be confirmed by LOH, quantitative PCR and fluorescence in situ hybridization (FISH). We were also able to detect deletions at the breakpoint region of 2 children with known translocations/inversions, which were not detected by cytogenetic investigations. Therefore, microarrays provide an efficient way to serve as a tool for precise mapping regions with allelic imbalances and chromosomal breakpoints and may help to characterize new microrearrangement syndromes, delineate regions of UPD and identify genes involved in chromosomal rearrangement phenotypes. Further studies will also be performed to determine the resolution of different SNP genotyping and tiling path array techniques.

C80. Annotation of the protein-coding genes in the ENCODE regions

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The aim of the ENCODE project (http://genome.gov/10005107) is ultimately to identify all the functional elements of the human genome. During its pilot phase, ENCODE focuses on 44 regions of the human genome corresponding to 1% of its sequence (Science, 2004, 306, 636-40). As part of this project, the GENCODE consortium by combining manual annotation with experimental validation, thus allowing constant refinements, produced a high quality annotation of the genome to be used as the "reference set" by the ENCODE consortium members.

To uncover further the complex architecture of the human transcriptome and potentially identify new gene elements (exons), we combined 5'RACEs with high-density 22 nucleotides-resolution tiling arrays. PolyA-RNA from twelve adult human tissues and three established human cell lines were analyzed to investigate the complexity of the human transcriptome expressed in the ENCODE regions. Indeed, the use of the RACE technology allows the detection of low copy number transcripts/isoforms and a high-resolution analysis of each gene in an individual manner but with high-throughput using pooling strategies. These experiments allowed the identification of as-yet unannotated exons and tissue/cell line specific, distal unannotated 5' exons in the majority of the 399 tested genes. A significant subset of these alternative distal 5' exon span large segment of genomic sequences away from the main portion of the coding transcript and often overlap with the next 5' positioned gene(s). These data are supported by independent experiments mapping the transcription start sites using CAGE and PET-Ditags.

C81. Island of euchromatic-like sequence and expressed genes within the short arm of HSA21: sequence and copy number variability.

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Although the sequence of the euchromatic portion of the human genome is essentially complete, the heterochromatic regions remain unknown. These regions include the short arms of the acrocentric chromosomes. Among these, the short arm of HSA21 (21p) has special significance because of its involvement in translocations resulting in trisomy21. We constructed a BAC library from the human-mouse somatic hybrid cell line WAV17, monoallelic for HSA21. We generated 1.3Mb of 21p sequence from 8 BACs. Surprisingly, 21p contains islands of sequence showing euchromatic-like features with an interspersed repeat content similar to that found on 21q. In silico and EST-based predictions identified 29 gene models, a third of which were shown to correspond to bona-fide genes by RT-PCR in 24 human tissues. We mapped the 5' ends of these transcripts by RACE and defined their structures. Analysis of these transcripts in different individuals shows extensive nucleotide variability and alternative spliced isoforms among different tissues suggesting multiple inter- and intrachromosomal copies. Moreover they map to the short arms of multiple acrocentrics as determined with monochromosomal cell hybrids. Quantification of their copy number by qPCR suggests that they are present in 4-50 copies in the human genome. Since the gene content of the heterochromatic regions of the genome appears to be underestimated, more efforts should be made towards the characterization of these unexplored regions. For this goal we have end-sequenced the entire BACs WAV-17 library and selected 47 clones that do not correspond to 21q for further sequencing.

Clinical genetics

P0001. Werner Syndrome In Three Members Of A Family Presenting As Hoarseness And Scleroderma

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Some of genetic disorders may be diagnosed enormously as some more routine diseases in clinical practice. This is true also for werner,s syndrome which may mimic scleroderma. Werner,s syndrome is a autosomal recessive disorder secondary to mutation in helicase gene (WRN) on short arm of chromosome 8. It typically begins with graying of hairs in second decade of life and skin changes , hoarseness , early cataract , leg ulcer and diabetes ensue before age 34 . Patients with werner,s syndrome are prone to a variety of malignancies and accelerated atherosclerosis.

Here in we present a 32 years old lady with werner,s syndrome which was under extensive evaluation due to hoarseness . Skin stiffening was the leading cause of referral to us. Constellation of symptoms along with family history of similar condition in her two sisters lead us to the of werner,s syndrome as the cause of condition .

P0002. New skeletal abnormalities in a case of Cardiofaciocutaneus Syndrome

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INTRODUCTION: Cardiofaciocutaneus-CFC syndrome is an autosomal dominant disorder, in that the manifestations included included macrocephaly; characteristic facial features; growth retardation; cardiac defect; sparse, curly hair; neurologic impairment/developmental delay; gastrointestinal dysfunction; ocular abnormalities/dysfunction; history of polyhydramnios; and hyperkeratotic skin lesions. CASE REPORT The propositus 10 years old male. At physical examination his height was 123 cm (-3 pc), weight 27kg (10-20 pc), OFC 56 cm (+97 pc). Clinically he showed short stature, macrocephaly, thick curly and sparse hair, peculiar facies characterized by blepharophimosis, sparse eyebrows, downslanted palpebral fissures, broad nasal bridge and short philtrum, short neck. The palmar and plantar regions shown hyperkeratosis and multiple palmar-plantar creases . The radiological examination revealed cuboid-shaped vertebral bodies and lack of lumbar lordosis, pelvis hypoplasia, short and broad tubular bones, brachymetacarpalia and brachymetatarsalia, brachydactyly of all fingers and hypertrophy of the 1st ray on hands and feet. elongated tumorous radiolucent defects in metaphyses of tibia bilateral. DISCUSSION: We describe a 10-year-old boy whose features were thought to satisfy the diagnosis of CFC syndrome and new radiologcal findings. CFC syndrome (MIM 115150) is distinguished from Noonan syndrome by the presence of abnormal hair and hyperkeratotic lesions and by its usual sporadic occurrence. Because CFC syndrome had been considered to be a more severe variant of Noonan syndrome, but not found abnormalities in the gene PTPN11 in CFC patients. The clinical diagnostic criteria be used in future studies aimed at identifying a molecular basis for this condition, and also differentiated CFC from Noonan and Costello syndromes.

P0003. The phenotype of three family members in two generations with an unbalanced 10;18 chromosome translocation: partial trisomy 10q and an 18q deletion syndrome

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We present a girl, 18 months of age, with narrow palpebral fissures, hypertelorism, epicanthic folds, a flat midface, an upturned nose, and a bow-shaped mouth. She is short (-2 SD) and has a toe position anomaly. Her development is slightly delayed. The pedigree revealed recurrent miscarriages. Cytogenetic analyses showed an unbalanced chromosomal translocation: 46,XX,der(18), t(10;18)(q26.1;q22.2). The breakpoints were confirmed by FISH analysis and MLPA. The father is a carrier of the balanced translocation t(10;18).

The carrier's brother, also a carrier, has a prematurely born son, known to have developmental delay and non-specific white matter changes on the MRI-scan of the brain. A second brother is institutionalized because of mental retardation. Both mentally retarded family members have the unbalanced translocation t(10;18).

The unbalanced family members share most of the facial dysmorphisms. All three had a short stature, toe anomalies and a vertical talus. The children have nystagmus and strabismus, no congenital heart defect. Their uncle can only speak a few words, had strabismus and a small cardiac septal defect, a retinal tear at age 7, has a high forehead and large lower jaw, hearing loss, scoliosis, and joint contractures of the fingers. Most of the phenotypic features fit best with the features of an 18q- syndrome, such as developmental delay, short stature, facial dysmorphism, foot deformities and eye movement disorders. White matter disease of the brain and epilepsy have been described in the 18q- syndrome. So far, epilepsy has not occurred in our patients.

P0004. 2q24.3 deletion syndrome: report of a case and review of the literature.

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We report a 3 years and 9 months old girl with postnatal growth retardation, microcephaly, down-slanting palpebral fissures, long eyelashes and micrognathia. Broad and long halluces with a wide gap between first and second toes were present. The other toes were remarkably short with hypoplastic phalanges. She also showed

developmental delay, seizures, lack of eye contact, stereotypic and repetitive hand movements and sleep disturbances with breath holding. Brain MRI showed an incomplete myelinization. Prenatal and postnatal (3 months) karyotype was normal. Array-CGH analysis revealed a "de novo" 2q interstitial deletion of about 10.4 Mb, involving segment between cytogenetic bands 2q24.3 and 2q31.1. The deletion was confirmed by quantitative PCR. A review of 52 children with interstitial 2q deletion reported in the literature identified 6 cases with a comparable deletion. The emerging phenotype includes postnatal growth retardation, developmental delay, mental retardation, microcephaly, and peculiar facial dysmorphisms. Long and broad halluces with wide space between the first and the second toes are present in all described patients.
The association of a 2q24-q31 deletion with digital anomalies is supported by the absence of significant digital anomalies in the vast majority of patients with a 2q deletion which does not overlap this region. These evidences suggest that bilateral digital malformations of hands and feet, including a wide gap between the first and the second toes, brachydactyly with/without syndactily, camptodactyly, and/or split foot, associated with other anomalies should represent a clinical hint for a deeper investigation of the 2q24-q31 region.

P0005. A rare case with 47, XXY karyotype and female phenotype

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Sexual development is a result of numerous events that occur in cells of various target tissues to induce morphogenesis of the reproductive system organs. Although the importance of the Y chromosome in male determination has been well established, at the genomic level, sexual differentiation depends on different genes such as the sex-determining and other genes. The SRY is responsible for the reproductive system morphogenesis and, primarily, directed differentiation of the bipotential gonads. The SRY gene is the most important in the genetic control of the male development and followed by the others. Sexual differentiation anomalies may exist a wide spectrum at birth. These include male and female pseudohermaphroditism, gonadal dysgenesis and true hermaphroditism. The development of molecular techniques has greatly contributed to clarify the process of sexual differentiation. In this study, we present clinical findings, conventional and molecular cytogenetics and molecular genetic findings of a phenotypicaly female case with 47, XXY karyotype.

P0006. Child with 4q Terminal Deletion and Mucocutaneous Candidiasis

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We report an eight-year old Saudi boy with subtle dysmorphic facial features, growth retardation, global developmental delay, cerebellar atrophy, and recalcitrant mucocutaneous candidiasis. Weight and height are less than 3rd centile, and OFC at 25th centile. The physical examination revealed subtle dysmorphic features with a high forehead, epicanthal folds and crowded teeth. The CNS examination was notable for generalized hypertonia and hyperreflexia. The patient has suffered from recurrent mucocutaneous candidiasis since the age of 18 months involving the mouth and nails with partial response to oral fluconazole. Recently, he has esophageal candidiasis based on esophageal biopsy, and barium enema showed significant gastroesophageal reflux. He has an improved response to oral voriconazole. Developmental assessment showed severe mental retardation and profound delay in gross and fine motor skills. Immunoglobulin levels, nitroblue tetrazolium test, HIV and leukocytic markers were normal. The blastogenesis revealed depressed lymphocytes' response to candida at 38% when compared to control. Diffuse cerebellar atrophy was found on MRI examination. Visual evoked potentials, electroretinogram and brain stem auditory evoked potentials were normal. The electroencephalogram showed diffuse background slowing and disorganization indicating diffuse cerebral dysfunction of a nonspecific nature, but no clinical seizure reported. Aside from the coxa valga, the skeletal survey was normal. Chromosome analysis of the patient revealed 46,XY,del(4)(q33). FISH using a 4p/4q subtelomere DNA probe assay, the finding confirms the deletion of qter subtelomere on chromosome 4. Parental chromosomes are normal. To our knowledge this is the first report of mucocutaneous candidiasis in a patient with 4q terminal deletion.

P0007. A new case of 7p duplication syndrome

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Introduction: Partial duplication of the short arm of chromosome 7 is a rare chromosomal disorder, which results in developmental, craniofacial, skeletal, and cardiovascular anomalies. There have been to our knowledge at least 58 confirmed cases of duplicated 7p which suggests a well-defined pattern of abnormalities.
Clinical Report: We report a case of a nine-month old female infant with a direct duplication of the 7p22.1-p13 chromosome region. The infant presented psychomotor retardation, stereotypic behavior, generalized hypotonia, and characteristic dysmorphic features. The weight, the length, and the head circumference were at 3rd, 25th, and 3rd centile correspondingly. The propositus presented a characteristic craniofacial appearance with a peculiar thin Wolf-Hirschhorn syndrome-like facies, high forehead, hypertelorism, slight palpebral fissures, pale highly arched eyebrows, thin lips, high narrow palate, micrognathia, straight and thin nose with a broad bridge, slightly angulated pointed tip and narrow inverted nostrils. The ears were low set and there were abnormal palmar creases. Skeletal anomalies included kyphoscoliosis, irregular form of the vertebrae, narrow thorax, and bilateral camptodactyly of the index finger. Traditional G-banding detected a partial 7p duplication, which was further demonstrated to be entirely of chromosome 7 origin by using a whole chromosome paint for chromosome 7 and derived from 7p22.1-p13 by multicolor banding (MCB) studies.
Discussion: Our observations in combination with other cases confirm that 7p trisomy due to dir dup(7p) can be regarded as a defined chromosome syndrome. FISH, when used, is essential in the confirmation of the cytogenetic abnormality and further delineation of the chromosomal disorder.

P0008. DNA-diagnostics of hereditary retinal dystrophies, caused by mutations in ABCA4 gene.

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Hereditary retinal dystrophies is a heterogeneous group of congenital retinal diseases, caused by degenerative changes in the photoreceptor cells of retinal pigment epithelium, with a severely reduced visual acuity outcome. At least four clinically polymorphic retinal dystrophies (Stargardt disease (STGD), fundus flavimaculatus (FFM), retinitis pigmentosa-19 (RP19) and con-rod dystrophy (CRD)) have been associated with mutations in the retina-specific transporter gene (ABCA4 or ABCR) related to the ATP-binding cassette superfamily. The DNA-analysis was performed for 24 unrelated patients clinically diagnosed with STGD, FFM, RP19 and CRD for the three most common ABCR mutant alleles: Gly863Ala, Ala1038Val and Gly1961Glu. Ala1038Val mutation was identified in ten patients: 2 patients with STGD were found to be homozygote and heterozygote; 2 patients with FFM were heterozygote and one patient were homozygote; 3 patients with RP19 and 2 patients with CRD were found to be heterozygote. Gly863Ala and Gly1961Glu mutations were not identified in any studied patient. The allelic frequency of the ABCR mutant allele, Ala1038Val, identified in 12 of 48 studied chromosomes, composed 25% in the group of patients has been investigated. The results of the research indicate a high effectiveness of elaborated DNA-diagnostics.

P0009. A new syndrome with abnormal gyral pattern, vermis hypoplasia, severe facial dysmorphism and cleft palate

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We describe the clinical, histopathological and molecular studies of a female proband that died at two months of age in the context of a new syndromic abnormal gyral pattern with vermis hypoplasia. There was no significant family history. Clinical and radiological features included poor contact, cleft palate, severe facial dysmorphic features with marked down-slanting palpebral fissures, retrognathism, fronto-temporal pachygyria, bilateral occipital polymicrogyria and vermis hypoplasia. A cytogenetic imbalance was ruled out using standard and high resolution chromosome analyses, Miller-Dieker and telomeric FISH studies and array-CGH. This description does not fit with any of the known syndromes with abnormal gyral pattern. The presence of vermis hypoplasia in this child permits to allows us this observation in the subgroup of lissencephaly with cerebellar hypoplasia. The severe clinical course of the disease, the severe dysmorphism and the absence of cerebellar hemisphere hypoplasia in this child rule out Norman-Roberts syndrome. Molecular analyses of the *LIS1*, *DCX, ARX* and *RELN* genes were negative. Consequently, the reported features in this child are unique and represent a new syndrome.

P0010. ABO blood group antigen on human uroepithelial cells responsible for pseudomonas aeruginosa infection

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Although the ABO blood group of the human host has been reported to influence infection in different part of human body, there have been few clinical observations on this effect especially in urinary tract. A cross sectional study was performed to investigate the relationship between blood group type and urinary tract infection induced by pseudomonas aeruginosa. Urinary tract infection was confirmed in 250 patients with pyuria and positive urine culture. In all 250 cases blood group were histochemically confirmed. Accordingly, clinical study has proved that urinary tract infection by P. aeruginosa can be positively correlated with blood group A. P aeruginosa apparently adheres specific to GalNAc as terminal carbohydrate of blood group A and indicate that patients with blood group A may have a genetic predisposition to UTI by P aeruginosa.

P0011. Acrocallosal Syndrome with temporal lobe hypoplasia

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Acrocallosal Syndrome is a rare genetic disorder which is characterized by moderate to severe mental retardation, agenesis or hypoplasia of the corpus callosum and polydactyly of fingers and toes. Although autosomal recessive inheritance has been suggested, acrocallosal syndrome usually seems to occur sporodically. The spectrum of this syndrome is very variable. Prominent forehead, broad nasal bridge, short nose and mandible, hypertelorism, epicanthic folds, large anterior fontanelle and tapered fingers, omphalacel and inguinal hernia are some other common findings in this syndrome. Twenty percent of the patients have accompanying brain abnormalities such as cerebral atrophy, hypothalamic dysfunction, small cerebrum, micropolygyria, hypoplasia of pons, hypoplasia of cerebellar hemispheres, hypoplasia of medulla oblongata, agenesis or hypoplasia of cerebellar vermis and corpus callosum abnormalities. Here we present a 10-month-old female infant with clinical and radiological findings indicative of acrocallosal syndrome. She was born to non-consangiuneous parents after a normal vaginal delivery. The family history and pregnant duration were unremarkable. She was noted to have craniofacial abnormalities suggestive of acrocallosal syndrome, optic atrophy and polydactyly. Magnetic Resonance Imaging revealed cerebral atrophy, corpus callosum agenesis, dilated lateral ventricule and unilateral temporal lob hypoplasia which the latter not having previously been reported in the spectrum of this syndrome before. From our finding we conclude the importance of screening brain abnormalities and present temporal lobe hypoplasia as a new additional anomaly in this syndrome.

P0012. A girl with aplasia cutis congenita, limb abnormalities and eye anomalies: severe Adams Oliver syndrome

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Adams Oliver syndrome (AOS; MIM 10030) is characterized by aplasia cutis congenita, most commonly of the scalp and skull, and terminal transverse limb defects. Other congenital anomalies such as congenital heart disease are frequently reported. Autosomal dominant inheritance with a great variability in expression and autosomal recessive inheritance have been suggested. No causal gene defect is known for AOS.

We report on a Turkish daughter of non-consanguineous parents. Pregnancy was complicated by oligohydramnion. At birth, she was microcephalic (OFC< -2SD) and had aplasia cutis congenita of the scalp and the upper abdomen. Her hands and feet had transverse reduction defects. An ultrasound examination of the cerebrum revealed bilateral cerebral calcifications, ultrasound examination of the abdomen showed her abdominal muscles underneath the aplasia to be hypoplastic. She developed a nystagmus in the first months of her life. Further ophthalmological examination revealed vitreo-retinopathy of both eyes, causing severe vision loss. At the age of 8 months her motor development was slightly delayed. Extensive chromosome analysis showed no abnormalities.

AOS has been suggested to be the result of a vascular disruption sequence. This could result in a spectrum of clinical findings, ranging from classical AOS to severe phenotypes, like in the proband presented here. This spectrum may well be caused by a single gene mutation.

P0013. Association of the DAT1 -67 T-Allele with Attention-Deficit/Hyperactivity Disorder (ADHD)

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Association between attention deficit hyperactivity disorder (ADHD) and the 10-repeat allele of a polymorphism (a 40 bp variable number of tandem repeats) in the dopamine transporter gene (DAT1) has been widely documented. In this study, we examined whether either allele of the DAT1 core promoter -67 polymorphism is associated with ADHD in a case/control study. The allele and genotype frequencies of the polymorphism were studied in 136 patients and 163 controls, which were matched on the basis of sex, age and ethnicity. The genotype frequencies in the patients group were as follows: AA 30.9%; AT 55.1%; TT 14% vs. the genotype frequencies in the control group: AA 49%; AT 41.8%; TT 9.2% [?2=10.3, df = 2, OR = 2.15 (95% CI 1.34-3.47, p = 0.006]. The T-allele of the -67A/T polymorphism revealed a ~1.4-fold excess in the patients group comparing with the controls (p = 0.003). 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. Replication studies of independent samples and family-based association studies are necessary to further evaluate the significance of our findings.

P0014. Evaluation of genetic services for Autosomal Dominant forms of Retinitis Pigmentosa and associated retinal disorders.

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The aims of this project are to evaluate genetic services for Retinitis Pigmentosa and related retinal disorders. The project develops a model for genetic services for other genetically heterogeneous disorders.

Retinitis Pigmentosa (RP) is a group of inherited progressive retinal diseases affecting about 1 in 3500 people worldwide. RP can be sporadic, autosomal dominant, autosomal recessive or X-linked. Currently we know of over 30 genes associated with this condition. Autosomal Dominant RP accounts for approximately 15-25% of all RP cases. We are currently developing testing for Rhodopsin (RHO) and Peripherin (RDS) which account for approximately 35% of cases of dominant RP. In addition we are investigating the involvement of a panel of common mutations and exonic hotspots across numerous RP genes in this group of patients. We are also evaluating testing peripherin in patients with a macular dystrophy.

The mutation detection rate in ADRP families so far is 28% of which 40% are Rhodopsin mutations, 30% are peripherin mutation and 30% are from the common mutation panel. The mutation rate in the macular dystrophy patients tested for RDS/peripherin mutations is currently 35%.Here we present the strategy and discuss the efficiency of the system to detect mutations.

P0015. Alopecia-Mental Retardation and Microcephaly in three Iranian siblings

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We are reporting 3 affected sibs, 2 girls, aged 13, and 7, one boy, aged 12 with total congenital alopecia, mental retardation, and microcephaly in an Iranian family. The parents are first cousins with no other pregnancies or offspring. The first child has refractory seizures, while the second has hypergonadotropic hypogonadism, and the third had neonatal teeth.

Their teeth, nails, sweating and hearing are normal. They are not dysmorphic. The skin biopsy of the second child showed agenesis of pilosebaceous apparatus.

Alopecia, epilepsy, and mental retardation OMIM 203600 are the findings in the Moynahan alopecia syndrome based on an initial report by Moynahan (1962), who reported two brothers with familial congenital alopecia, epilepsy, mental retardation and unusual electroencephalograms. Later, Mosavy (1975) observed 4 affected sibs and Pfeiffer and Volklein (1982) reported an affected brother and sister with microcephaly and no seizures. Seizures was present in the reports by Wessel et al. (1982) in 3 sibs and in the 2 affected boys of family with affected fathers and 3 children reported by Van Haeringen (1990).

Our cases have all of the above findings and would suggest that the syndrome is heterogeneous with various clinical manifestations or variable expressivity. Our second patient has hypergonadatrophic hypogonadism which has been reported in the cases of Pridmore et al. OMIM 601217 where the affected individuals have alopecia, mental retardation, seizures, and hypergonadotropic hypogonadism.

P0016. Alpha-1-antitrypsin deficiency in Iranian population: Mutation Detection

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Alpha-1-antitrypsin deficiency (AATD) is a hereditary autosmal recessive disorder, due to mutations in the alpha-1-antitrypsin (AAT) gene of 12.2 kb located on the chromosomal segment 14q31-32.3. Alpha-1-antitrypsin deficiency effects mainly the lung and liver leading to neonatal cholestasis,chronic hepatitis or cirrhosis. Antitrypsin deficiency is widely known in Europe as a disease of white population, who are at the highest risk for liver and/or lung disease. We investigated wether the AAT deficiency is a very rare or is a widely under-diagnosed disease in Iranian population.

85 unrelated children clinically characterized by idiopathic liver dysfunction suspected to AATD , genotyped for the Z,Smutations using PCR-RFLP method.Any other possible mutations in exons III and V were studied in affected individuals using PCR-SSCP method, followed by sequencing.

In order to estimate the frequency of Z and S alleles in Iranian normal population, 200 control individuals were screened for Z and S mutations.

The results suggest rare frequency of AAT gene mutations in Iran.

P0017. Determining different Alpha-Thalassemia deletions in Iran using Gap-PCR technique.

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Objective: we studied the prevalence of four most common α-thalassemia deletions in couples attending to this center in a 3.5 years period. **Background:** Thalassemias including α and β thalassemias are most common inherited single-gene disorders around the world. Fetuses with Hb Barts' hydrops fetalis caused by the homozygous α^0 thalassemia (--/--) die either in utero or shortly after birth. **Materials and Methods:** We have studied prospective couples presented with unusual hematological indices. Genomic DNA was extracted from peripheral blood. Gap-PCR was used to screen most frequent deletional mutations causing α-thalassemia (i.e. $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-(\alpha)^{20.5}$, $--^{MED}$) .**Results:** Two hundred thirty eight cases with hematological indices suggesting α-thalassemia were screened for the α-globin gene deletions. A total of 156 individuals (65.5%) presented with at least one of these deletions. From these, seventy six cases (48.7%) were heterozygote for $\alpha^{3.7}$ deletion ($-\alpha^{3.7}/\alpha\alpha$) and 50 (32%) were homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$). Twelve cases (7.6%) were $-(\alpha)^{20.5}$ and ten cases (6.4%) showed $--^{MED}/\alpha\alpha$ genotype. Also, eight cases (5.1%), were carriers of $-\alpha^{4.2}$ mutation ($-\alpha^{4.2}/\alpha\alpha$). **Discussion:** As it was shown above, deletions were detected in 156 out of 238 cases. Even if all 238 cases bear mutations in α globin gene, our results still showed similar results to others and deletions consists majority of the α globin gene mutations. Like other studies, $-\alpha^{3.7}$ was the most prevalent single gene deletion in Iranian population. However, $-\alpha^{4.2}$ has very less prevalence than other deletions.

P0018. Mutational analysis of the *DJ-1* gene in sporadic patients with amyotrophic lateral sclerosis

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BACKGROUND: Homozygous mutations in the *DJ-1* gene have been reported in an Italian family with three brothers affected by early-onset parkinsonism, dementia, and amyotrophic lateral sclerosis. OBJECTIVE: To assess the role of *DJ-1* among more common forms of amyotrophic lateral sclerosis (ALS). METHODS: DNA from 70 unrelated sporadic ALS patients of Italian origin was screened for mutations in *DJ-1* by direct sequencing of polymerase chain reaction-amplified fragments. All 7 exons of *DJ-1* and splice sites were screened. Each new variant identified was also analyzed among Italian controls. In one case carrying a novel heterozygous mutation, cDNA analysis was performed on RT-PCR material obtained from a muscle biopsy. RESULTS: two novel *DJ-1* heterozygous variants were detected. The missense p.A179T substitution was identified in ALS patients (2.8%) and controls (0.8%), and is therefore unlikely to be pathogenic. The missense p.R156Q mutation was detected in one patient (1.4%), but in none of 580 control chromosomes. Although cDNA analysis revealed no evidence for a second mutation in this patient, a pathogenic role for the p.R156Q mutation cannot be excluded. CONCLUSION: Mutations of *DJ-1* gene are not a common cause of sporadic ALS in this Italian sample; further studies are needed to clarify the contribution of *DJ-1* mutations in common forms of ALS, especially in the familial cases.

P0019. Segmental aneuploidies in patients with epilepsy of unknown etiology detected by array-CGH

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Background: Epilepsy and epileptic EEG abnormalities are common symptoms or signs of specific chromosomal abnormalities. This indicates that these chromosomal regions harbor genes involved in epilepsy or epileptogenesis. Submicroscopic segmental aneuploidy detection by array-CGH has been applied extensively in patients with unexplained mental retardation ascertained through clinical checklists. We used a similar checklist to score patients with epileptic disorders and applied array-CGH in the first 16 patients.

Methods: In a cohort of 675 pediatric patients that underwent an EEG-examination at the University Medical Centre Utrecht, 35 patients were selected for analysis by array-CGH. Inclusion criteria were refractory epilepsy of unknown etiology, mental retardation and at least one of the items described in the 5-item checklist developed by de Vries and coworkers. Array-CGH was applied to the first 16 patients with scores ranging from 1-5 items.

Results: Four out of 16 (25%) patients analyzed by array-CGH showed segmental aneuploidies, including duplications involving chromosomes 9 and 16, and combinations of deletions and duplications involving chromosomes 3 and 4. Two of those aberrations were suspected after standard karyotyping, while the other two aberrations were found in patients with a normal karyotype. All four patients had a score ≥ 4 on the clinical checklist, whereas no abnormalities were found in patients scoring ≤3.

Conclusions

1. We demonstrate the added value of array-CGH by detection of submicroscopic segmental aneuploidies in patients with epilepsy of unknown etiology.
2. We show that a clinical checklist is useful in preselecting candidate patients with epilepsy for analysis by array-CGH.

P0020. Angelman syndrome with a new mutation and hyperamoniemia

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We present a seven year old boy born from second pathological pregnansy, first child of unrelated parents. He is born at fourty weeks, gestation by spontaneous delivery after an uneventful pregnancy. Birth weight 3 350g and length 50 cm. Since the first month of his birth the child is with jerky movements. During early pregnancy the parents noticed periods with laugh frequently for almost any reason. At age of 20 months started seizures. Due to that fact began treatment with Depakin. At three years was detected high ammonia level. Because of that the therapy was changed but the hyperammoniemia still existed and is the reason for low protein diet.

The child has specific face dismorphism- happy disposition, microcephaly, macrostomia, protruding tongue, open mouth, and widely spaced teeth, prognathism. Specifically, ataxia-like incoordination, hyperkinesia, hyperactivity, restlessness, jerky movements, ataxic gait or complete inability to walk and absent speech, sleeping disorders and feeding problems are the main symptoms. The principal neurological disorders are epilepsy and EEG abnormalities - a slow wave of spike activity and hypsarrhythmia. The child is mentally retarded.

DNA analysis - Methylation-specific PSR of the SNRPN gene- normal parents fragments. Mutation analysis of UBE3A gene- L747P mutation in exon 14 was found in both the child and his mother. This is the first time this mutation is described with this clinical picture.

The methylation tests can fail to detect some familial Angelman syndrome cases with a recurrence risk of 50%.

P0021. Rhizomelia with anal atresia and anophthalmia; is it a new syndrome?

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Clinical findings were identified in a newborn with multiple parental consanguinity. Dysmorphic features included anal atresia, anophthalmia, short stature, rhizomelia of upper and lower limbs, hypoplastic nails, small-dysmorphic ears, short neck and various heart defects including dextromesocardia, atrial isomerism and insufficiency of right atrioventricular valve. The radiological findings showed shortness of proximal limbs. The infantexpired soon after birth. In addition, the postmortem examination revealed bifid sternum, thymic hypoplasia, annular pancreas with congenital cardiac defects. The case had overlapping features with coditions such as CHARGE, VACTERL, Fraser-cryptophthalmos, Lenz microphthalmia and fetal thalidomide syndrome but the concurrence of rhizomelia with anal atresia and anophthalmia has not been described previously. Three sibs of the father were also born to consanguineous parents with anophthalmia and died soon after birth. This supports the possibility of a new autosomal recessive inherited condition.

P0022. Presentation, natural history and management of aneurysm syndromes caused by mutations in *TGFBR1* or *TGFBR2* encoding for transforming growth factor-β receptors

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Loeys-Dietz syndrome (LDS) is a newly defined autosomal dominant aortic aneurysm syndrome, characterized by the triad of arterial tortuosity/aneurysms, hypertelorism and bifid uvula/cleft palate, and caused by heterozygous mutations in the genes coding for transforming growth factor-beta receptor 1 or 2 (*TGFBR1* or *TGFBR2*).

We describe the clinical, molecular characterization and natural history of 50 families. Thirty-eight probands presented with typical manifestations of LDS. In view of the phenotypic overlap between LDS and vascular Ehlers-Danlos syndrome (EDS), we screened an additional cohort of 40 patients with a typical presentation of vascular EDS in the absence of the type III collagen abnormalities and the craniofacial features of LDS.

A mutation in *TGFBR1* or *TGFBR2* was identified in all studied typical LDS probands (LDS-I) and in 12 probands with vascular EDS-like presentation (designated LDS-II). The natural history in both groups is characterized by aggressive arterial aneurysms (mean age at death 26.1 years) and high incidence of pregnancy-related complications including death and uterine rupture (6/11 pregnant women). Individuals with LDS-I showed earlier cardiovascular surgery (13.0 vs. 26.9 years) and death (22.1 vs. 31.8 years) compared to LDS-II. There have been 54 vascular surgeries in this cohort of patients with only one example of intra-operative mortality, a clear distinguishing feature from vascular EDS. Mutations in either *TGFBR1* or *TGFBR2* predispose to aggressive and widespread vascular disease and a substantial rate of pregnancy-related complications. Clinical presentation is predictive of outcome. Genotyping of individuals may guide therapy including the use and timing of prophylactic vascular surgery.

P0023. No Genetic Variation Of ARG1 are involved In Persistance Of Fetal Hemoglobin In Sickle Cell Disease Patients

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Sickle Cell Disease (SCD) is influenced by foetal haemoglobin (HbF) concentration. In SCD patients HbF production is under tight genetic control and varies over a 20-fold range. A genetic entity so-called Heterocellular Persistance Of Fetal Haemoglobin was defined in patients where high level of erythrocytes containing HbF leads to fewer pain episodes and longer survival. Located in 6q22.3-23.1 the

HPFH locus comprises genes among which figures ARG1. Arginase catalyzes arginine hydrolysis to ornithine and urea. As L-Arginine is the nitrogen donor for NO synthesis, SCD induced hemolysis will release erythrocyte ARG1 and limit arginine plasma and cellular concentration then NO availability. Combined with HbS release that will stronger scavenge NO than normal hemoglobins, both mechanisms contribute to pulmonary hypertension, the major SCD complication.
To study ARG1, we collected DNA samples of 40 SCD patients (10 with HbF 1% and <20%, 10 with HbF >20%) without genetic modification known to increase Hbf concentration and 10 healthy controls. We then tested loss of heterozygoty using fluorescent markers (D6S976, D6S626, D6S270) and studied the 8 coding exons, their flanking intronic sequences, the 5'UTR and 3'UTR of ARG1 by sequencing. No genetic defect was found.
We have excluded here an ARG1 gene variation as a major causative event of HbF overproduction in SCD patients. As no other known gene in the HPFH locus seems to be involved in HbF production, it will be of great interest to investigate new ORFs with unknown function pointed out in this region.

P0024. Array CGH characterization of patients with Retinoblastoma and associated malformations.

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We report 3 patients with retinoblastoma, dysmorphic features and developmental delay. Case 1 is a 2 y/6 m old male with high and broad forehead, deeply grooved philtrum, thick everted lower lip, and thick anteverted lobes. He also has a prenatal growth retardation, iris heterochromia, and short fifth toe. Unilateral retinoblastoma was diagnosed at the age of 10 months. Case 2 is a 1 y old female with similar facial features except for thick lower lip. In addition, she has dolico-macrocephaly, micrognazia, ephicantic folds and toe crowding. Bilateral retinoblastoma was diagnosed at the age of 5 m. Case 3 is a 8 y old female with moderate growth retardation, severe microcephaly, thick lower lip and ephicantic folds. At the age of 2y/5m was diagnosed by unilateral retinoblastoma. Whole genome array CGH analysis demonstrates a de novo 13q deletion of different size in case 1 and case 2. On the contrary, case 3 has a 200Kb deletion on chromosome 7q (7q11-21) inherited from the mother. A 75 Kb resolution array CGH analysis was unable to identify other genomic deletion. Germ-line RB1 point mutation analysis was negative. Our results confirm that there is a distinct facial phenotype related to 13q deletion contigous gene syndrome characterized by high and broad forehead, deeply grooved philtrum and thick anteverted lobes. Patients with retinoblastoma and other malformations without a distinct facial phenotype may have a different contigous gene deletion syndrome or a casual association of mental retardation and retinoblastoma due to a somatic RB1 gene mutation.

P0025. Genetic aberrations and its association with recurrent ART failure

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Genetic aberrations may result poor blastocyst development, implantation failure and failure of *InVitro fertilization* (IVF). Assisted Reproductive Technology (ART) has revolutionised the management of infertility and allows infertile couples to procreate.
Genetic analysis was done in 265 infertile males and 30 couples going in for IVF. Chromosomal abnormalities were found in 46 infertile males. We found 26 cases with Klinefelter Syndrome (KFS), 28 cases were KF mosaics and 8 were mosaic variants, three cases with 46,XY 1qh+ and two case with 46,XY16h+. and five cases with robertsonian translocation. In 5 of the 30 couples opting for ART genetic analysis in the female partner revealed 46,XXq- chromosomal complement in two cases and Yq microdeletion in the AZFc region in 2 cases and one case had deletion of AZFa,b and c loci. Deletion of long arm of X chromosome(Xq-) in the female partner might have resulted in repeated failure of blastocyt development. This couple had gone in for 4 IVF cycles which had failed . The male partner was cytogenetically normal and had no Yq microdeletion . In cases with sex chromosomal and autosomal aberrations there is probability of poor embryo development and consequently poor implantation , which may be a result of high segregation abnormalities and may negatively affect the outcome of assisted reproductive techniques. Thus the presence of genetic anomalies results in poor IVF outcome and vertical iatrogenic transmission of these anomalies through ART.

P0026. ATRX Syndrome in a girl with a heterozygous mutation in the *ATRX* Zn finger domain and a totally skewed X inactivation pattern

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Mutations in the X-encoded gene *ATRX* are known to give rise to syndromic mental retardation in male patients whereas carrier female patients usually present a skewed X-inactivation pattern leading to an asymptomatic phenotype. Here, we describe a 4 years old girl with typical features of ATRX syndrome, carrying the recurrent R246C mutation of *ATRX*. Surprisingly, the X-inactivation pattern was totally skewed and the activated X chromosome, which proved to be maternally inherited, was the one carrying the *ATRX* mutation. To our knowledge, this is the first ATRX syndrome case reported to date in a female patient. Since this girl was born after *in vitro* fertilization, we discuss the possible responsibility of assisted reproduction technologies in the unexpected methylation pattern of her X chromosomes.

P0027. Clinical and molecular characterizationof a case of autism associated with an interstitial 1q deletion (1q23.3-24.2) in a patient with a *de novo* apparently balanced 1;5 translocation

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Reciprocal translocations represent one of the most common structural rearrangements observed in man, with an estimated prevalence ranging from 1/673 to 1/1000. They usually are inherited, but can also occur as *de novo* mutations, that are much rarer entities.
We report on a male patient showing developmental delay, minor dysmorphic features and autism. Standard cytogenetic analysis revealed that he carried a *de novo* apparently balanced translocation, t(1;5)(q23;q22). Searching for cryptic chromosome abnormalities, YAC clones in the region of the chromosomal breakpoints were selected and used as FISH probes. The patient showed a deletion in chromosome 1 from q23.3 to q24.2, with a loss of about 8 Mb. In an attempt to further characterize the deletion, with the aim to delineate a better genotype-phenotype correlation, whole genome screening was conducted using array-based CGH analysis, at a resolution of 75 kb. The array CGH data confirmed a cryptic deletion in the q23.3 to q24.2 region, without other imbalances. The breakpoints were better refined and the size of the deletion was evaluated in 4.97 Mb.
When an individual carries an apparently balanced *de novo* rearrangement, the risk for phenotypic abnormalities is significantly higher than for an individual who has inherited a similar rearrangement from a normal parent. A number of different mechanisms can be responsible for the abnormal phenotypes. In our patient the clinical picture is most likely caused by deletion of one or more genes in 1q23.3-->q24.2, a region of rising interest in the research for autism susceptibility genes.

P0028. Ring chromosome 17 in a girl with autism

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A mosaic of ring chromosome 17 and chromosome 17 monosomy was found in a girl with neurofibromatosis, mild dysmorphic features, growth and mental retardation, and atypical autism. The ring chromosome was further analysed using fluorescence in situ hybridisation (FISH) and multiplex ligation-dependent probe amplification (MLPA) of subtelomeric regions. The classical cytogenetics mapped the breakpoints on both arms of the ring chromosome to the terminal G-bands. The molecular methods showed the absence of both subtelomeric loci but presence of the MDLS region on 17p. Therefore, the extent of the deletions must be between 0.6-2.5 Mb on 17p, and 0.6-10 Mb on 17q. Interestingly, the girl meets the NIH criteria for neurofibromatosis. Based on this and on a literature review we argue that in addition to the universal “ring syndrome” which is based on ring instability and is less specific for the chromosome involved, various ring chromosomes may underlie their own characteristic phenotypes. In our patient the symptoms of neurofibromatosis could be attributed to the mosaic hemizygosity for the NF1 gene in some of her somatic cells. Several candidate loci for autism have been mapped to chromosome 17, and mosaic hemizygosity or direct involvement of respective genes in the aberration could possibly influence also this facet of the phenotype of our proband. It is a question if the chromosome 17 monosomy in a substantial fraction of her somatic cells can also have consequences for other future risks, for example due to her mosaic hemizygosity for the BRCA1 and TP53 genes.

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P0029. Phenotypic and genetic characterization of a family with autosomal dominant autoimmunity

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Autoimmune diseases result from dysfunctions during development and maintenance of self-tolerance and affect 3-5% of the general population. Apart from rare monogenic forms such as APECED and IPEX the etiology is multifactorial. We describe a nonconsanguineous German family with more than 25 members over 4 generations 5 of whom suffer from autoimmune diseases. Of those 3 females were diagnosed with type 1 diabetes (T1D) and autoimmune thyroiditis (AT), 1 female with T1D, AT, and celiac disease (CD), and 1 female with AT. The age of onset ranges from 8 months to 35 years. Extensive serologic investigations revealed the presence of autoantibodies specific for Addison´s disease, primary biliary cirrhosis, pernicious anemia, T1D, AT, and CD among affected as well as 5 additional family members without apparent clinical disease. Candidiasis, hypoparathyroidism, and mutations in the AIRE-gene were absent excluding the diagnosis of APECED. Thus, the findings in this pedigree are consistent with autosomal dominant autoimmunity resembling autoimmune polyendocrinopathy type 2. Since organ-specific autoimmune diseases are T-cell mediated we carried out gene expression profiling in cultivated T-cells from 2 affected and 2 normal controls using the HG-U133 microarray. We found a number of differentially expressed genes involved in chemokine activity, signal transduction, and proliferation suggesting a pathogenic role of impaired T-cell function. Currently, we are carrying out whole-genome linkage analysis to map the disease locus. Identification of the gene causing this form of autoimmune polyendocrinopathy may shed light onto the pathogenesis of more common forms of autoimmune disease such as T1D and AT.

P0030. Two novel mutations in the ELN gene in patients with autosomal dominant cutis laxa and systemic manifestations

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Autosomal dominant cutis laxa (ADCL) is a rare connective tissue disorder, characterized by generalised loose skin folds. Apart from mild pulmonary emphysema, the condition has historically been considered as a strictly dermatological disease. However, recently aortic root dilatation was reported in 4 patients from two families. ADCL is caused by frameshift mutations at the 3’ end of the elastin gene (*ELN*), resulting in an extended protein. So far, only 7 mutations have been reported.
We performed direct sequencing of the 3’end of the *ELN* gene (exons 28-34) and found two previously unpublished *de-novo* mutations in exon 33, c.2278_2281dupGCAG and c.2315delC respectively, in two patients with generalised cutis laxa. Light- and electronmicroscopic examination of their skin biopsies showed pronounced rarefaction of elastic fibres as well as diminished and disorganised elastin deposition. Importantly, both patients presented progressive aortic root dilatation and mitral valve insufficiency at respectively 3 and 11 years old. Interestingly, the latter also had a bicuspid aortic valve and a dilatation of the ascending aorta. It is likely that the presence of the bicuspid aortic valve aggravated the aortic enlargement due to the deficient elastin. Moreover, she also presented severe pulmonary emphysema (rest volume 208%; Tiffeneau index 40.4%).
Our data contribute further evidence that ADCL is a systemic disease with cardiovascular and pulmonary complications. Progressive aortic dilatation underscores the need for regular echocardiographic follow-up. We are currently investigating the mechanism underlying this aortic aneurysm formation.

P0031. Type II autosomal dominant osteopetrosis in three asymptomatic brothers caused by a mutation in the CLCN7-gene.

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Osteopetrosis is a genetic disease characterized by an increase in bone density due to impaired bone resorption. Autosomal recessive and autosomal dominant forms exist. Autosomal dominant osteopetrosis (ADO) type II, is characterized by a generalized osteosclerosis, predominantly involving the spine (Rugger-Jersey spine), the pelvis (“bone-in-bone” appearance), and the skull base. ADO type II has variable clinical expression and incomplete penetrance and is caused by heterozygous mutations in *CLCN7*.
Here, we report on three asymptomatic brothers (one twin-brother) with ADO type II, who showed a heterozygous missense mutation (R767W) in *CLCN7*, previously described in 3 other families with variability in clinical phenotype. Radiologically, we found increased bone density in the skull in 2 brothers, Rugger-Jersey spine and increased pelvic bone density in all three brothers (bone-within-bone appearance in 1 brother). In one brother bone scintigraphy showed increased uptake in humeri, left SI joint and distal femurs. Functional studies showed that osteoclast generation from peripheral blood of one brother was normal and that these osteoclasts were able to polarise and form acidic and TRAP containing vesicles, but had markedly reduced resorptive capacity compared to controls. By transmission EM the osteopetrotic osteoclasts showed rudimentary ruffled borders and multivesicular bodies, which are associated with uptake of resorbed matrix. However, they contained increased numbers of secretory vesicles, which is associated with reduced resorption, as described in other types of osteopetrosis. The in vitro data support the clinical picture, demonstrating normal osteoclast formation coupled with reduced, rather than abolished, osteoclast function in individuals with this mutation.

P0032. Genetic analysis in men with azoospermia - IVF-MESA/ TESE candidates

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We performed complex genetic counselling in 45 men with azoospermia - candidates for IVF-MESA/TESE.
We performed the routine genetic counselling, pedigree analysis, cytogenetic analysis and DNA analysis of the CFTR gene (mutations F508del, dele2,3(21kb), G542X, G551D, R553X), analysis of the 5T alele in intron 8 CFTR gene and analysis of deletions in AZF region (Yq).
In 5 of our patiens we found serious health complications. In 3 men we found pathological karyotype. We detected genotype CFTR F508del/ F508del in one patient, in 4 patients we found one pathological mutation in CFTR gene and in 4 men the 5T alele in intron 8 CFTR gene.The deletion in AZF region we found in two men.
Our analysis detected pathological findings in more then 25% of the patients.
We recommend complex genetic counselling in men with azoospermia before planning of methods of assisted reproduction. Our departement would offer genetic counselling and precise prenatal or pre-implantation genetic diagnostics in most of these families.

P0033. Genetic diagnosis in a foetus with Baller-Gerold syndrome

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We report on a foetus of 23 weeks, diagnosed with Baller-Gerold syndrome.
After a second pregnancy in which serious malformations on ultrasound were present again, a genetic diagnosis could be made.
The parents were healthy and there was no consanguinity.
The first pregnancy was interrupted at 23 weeks because of serious malformations : craniosynostosis, bilateral short forearms with curved ulnae and absence of the radius and the thumbs. A tentative diagnosis of Baller-Gerold syndrome was made.
A second pregnancy was interrupted ad 11,5 weeks because of following abnormalities : bilateral absence of the thumbs, oligodactyly of the hands and feet, short limbs and absence of one kidney.
Diagnosis of VACTERL, Roberts syndrome, Saethre-Chotzen and Fanconi were considered but had to be rejected.
Mutational analysis of the RECQL4 gene led to a genetic diagnosis.

P0034. A large Turkish Bazex - Dupre - Christol syndrome family

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Bazex - Dupré - Christol (BDC) syndrome is characterized by hypotrichosis , generalized state of atrophoderma , multiple milia of face mostly disappearing with puberty and development of basal cell carcinomas mainly affecting the skin and mucosa. It was first described in 1964 by Bazex in six members of a family. In 1977 Viksnins suggested X - linked dominant inheritance based on the fact that there was no male to male transmission in all the pedigrees published. Although several other large families have been reported since 1964 with approximately 120 affected members from 16 families , BDC syndrome can still be considered as a rare genodermatosis. In 1995 , Vabres et al. obtained evidence in three families for assignment of the gene to the Xq24- 27 region.
We here report- to the best of our knowledge - the largest BDC family with 29 affected members in four generations. Pedigree showed no male to male transmission supporting X - linked inheritance. In an attempt to identify the responsible gene , we first aimed to eliminate any candidate genes located in the BDC critical region . Therefore , NDUFA1 gene encoding an accessory protein identified in mitochondrial complex 1 , downregulated in basal cell carcinoma , was selected and excluded by sequence analysis in three affected family members. Further studies are planned to perform chromosome X wide linkage analysis to map the disease causing gene.

P0035. Brachydactyly type A1 manifestations in a 22-year-old girl with monosomy 5p13.3→pter

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Two gene loci are known so far for brachydactyly type 1 (BDA 1) a Mendelian disorder characterized by shortened or malformed digits, short metacarpals, metatarsals and others. One such locus was localized on 5p13.3-5p13.2 by linkage analysis, therefore we can expect such abnormalities in monosomy of a long segment of 5p with 5p13.3-5p13.2 included.
We present the natural history of a 22 years-old girl with monosomy 5p13.3→pter with many classical for Cri du chat syndrome traits and skeletal findings belonging to brachydactyly A1 syndrome spectrum with changes in hands and feet such as: as clinodactyly of 5th fingers, short 4th metacarpals, small, broad feet, short, broad toes (especially in 3rd, 4th and 5th toes of right foot) and scoliosis.
It is worth to notice, that the recently mapped ADAMTS12 gene at 5p13.3 corresponds to breakpoint position at 5p13.3 of presented patient. The possible role of deletion of ADAMTS12 gene or two other candidate genes CDH6 and NPR3 should be considered in pathogenesis of occurence of skeletal changes observed in presented girl.

P0036. Benign familial neonatal convulsions: clinical and genetic analysis in seven Dutch families

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Background: Benign familial neonatal convulsions (BFNC) is an autosomal dominantly inherited 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).
Methods: Analysis of the KCNQ2 gene was performed in seven families and linkage analysis in one.
Results: In four families, four different mutations were detected; one nonsense mutation (c.1756C>T, p.Gln586Stop), one missense mutation (c.1076C>A, p.Thr359Lys) and two frameshifts (c.1229delC, p.Pro410fs; c.1601delC, p.Pro534fs). The missense mutation was found in a proband and her affected mother, but not in the unaffected grandparents, indicating a new mutation. In a fifth large family, linkage analysis excluded the KCNQ3-locus, but screening of the KCNQ2-gene did not reveal a mutation. The proband was homozygous for a polymorphism (c.2339A>C, p.Asn780Thr) in this gene, while her daughter did not carry this allele. This indicated a large deletion of the KCNQ2-gene, to be confirmed by MLPA-analysis.
Conclusion: Five different mutations (71%) in the KCNQ2-gene were detected in seven families with BFNC. This confirms that the KCNQ2-gene is a major locus for BFNC, also in the Netherlands. Our findings illustrate that mutation analysis is also relevant in small families and, probably, sporadic cases, and should include testing for large deletions.

P0037. Bilateral perisylvian polymicrogyria in combination with cleft palate. Connection or coincidence?

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Bilateral Perisylvian Polymicrogyria (BPP) is a condition characterized by a specific combination of clinical signs and radiological features. This syndrome should be suspected clinically in infants presenting with mild developmental delay, epilepsy and pseudobulbar palsy. However, the diagnosis is based upon the typical MRI features, which consist of the bilateral presence of polymicrogyria in the area surrounding the sylvian fissures.

Both non-genetic and genetic causes have been described. In 2002 Villard et al. mapped a locus for BPP to the distal long arm of the X chromosome (Xq28).
Here we report on a woman who presented with speech disturbances and submucous cleft palate. History showed that she had been born at term to non-consanguineous parents. In the following years she experienced a delay in speech development. At the age of three a submucous cleft palate was discovered which was closed at the age of seven. Two years later, a pharyngoplasty was performed. When she was eight years old, she was referred to the child neurologist because of persisting speech disturbances, consisting of dysarthria in combination with impaired buccal, lingual, mandibular and swallowing functions. Evident pseudobulbar pathology was noticed. With the use of MRI, bilateral perisylvian polymicrogyria was recognized at the age of twenty-six. To our knowledge the combination of cleft palate and BPP hasn't been described before. The question remains if there is a link between BPP and cleft palate.

P0038. Presentation of a case with autosomal dominant hypospadias and biliary atresia polysplenia syndrome

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Biliary atresia is the most frequent cause of neonatal cholestasis and the most common indication for liver transplantation in children. It is associated with congenital, particularly splenic malformation in approximately 25 % of cases. Less commonly annular pancreas, intestinal malrotation, preduodenal portal vein, situs inversus, absent inferior vena cava, anomalous hepatic arteria and cardiac defects may also be components associated with this clinical status. Hypospadias is another congenital anomaly which occurs in 0.3-1/100 of male newborns. Autosomal dominant and autosomal resesive forms of isolated hypospadiases have been reported. But there is a multifactorial cause in most cases. Other malformations related to hypospadias are present in about 15% of infants with hypospadias however the combination of this anomaly with biliary atresia polysplenia syndrome (BAPS) has not been reported in the literature to date. Here we present a 6-month-old male infant who has biliary atresia, polysplenia, absent vena cava inferior, double collecting system, atrial septal defect and hypospadias. Pedigree analysis also revealed hypospadias in the father without other features of BAPS.

P0039. Genetic studies of bipolar disorder in Iran

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Although genetic factors have an important role in the etiology of bipolar disorders, but no specific gene has been conclusively identified, to be related to this disorder. Now, the investigators are studying on several genes may be related to bipolar disorder. One of the most important genes that surveyed is MAOA gene that codes MAOA enzyme, such changes, can lead to manifestation of symptoms of mood disorders. In this study , 100 patients with bipolar type I disorder and 100 healthy subjects from Iranian population have been selected and studied for three polymorphic markers of MAOA gene including the MAOA-CA repeat , the MAOA restriction fragment length polymorphism (RFLP) , and a repeat directly adjacent to the variable number of tandem repeats (VNTR) locus. There was no significant difference between cases and controls in RFLP results. The results regarding to MAOA and VNTR are shown in tables 1, and 2. The results show that in Iranian population allelic distribution is similar to other regions of the world, but the type of alleles related to this community is different from others.

Table 1: MAOA allele frequencies in the studied groups

Allele	Case	Control	P value for case-control
a0	26	17	**0.066**
a1	7	13	0. 251
a2	10	12	0.849
a3	14	11	0.371
a4	16	25	0.250
a5	14	24	0.165
a6	22	18	0.305
a7	28	30	0.885
a8	13	15	0.913
a9	5	8	0.509
a10	1	-	0.292

Table 2: VNTR allele frequencies in the studied groups

Allele	Case %	Control %	P value for case-control
V1	12.2	18.8	0.103
V2	48.1	49.1	0.856
V3	8.3	14.5	0.082
V4	31.1	16.4	**0.002**
V5	--	1.2	0.168

P0040. Post-axial polydactyly in a child with Branchio-Oculo-Facial (BOF) syndrome

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BOF syndrome (OMIM #113620) is a rare autosomal dominant disorder with highly variable expression. Typical are the cervical branchial sinus and infra-auricular skin defects which can include remnants of thymic tissue. Craniofacial findings include sparse hair, hypertelorism, upslanting palpebral fissures, flattened nose tip and malar hypoplasia. A majority of patients has 'pseudoclefting' of the upper lip. Upper lip pits are seen in some patients. Ocular findings include coloboma, nasolacrimal duct stenosis/atresia, microphthalmia and anophthalmia. Ears may be low-set with uplifted earlobes. Developmental delay, mental retardation, pre- and post-natal growth retardation and pre-axial polydactyly (n=1) has been described. A gene locus for BOF syndrome has not yet been identified.Here we describe a Dutch girl with BOF syndrome. She is the fourth child of non-related parents. Pregnancy and delivery were uneventful. Birth weight, length and OFC were all -2 SD. She has a pseudocleft, an upper lip pit, small bilateral retro-auricular skin defects, upslanting palpebral fissures, hypertelorism, a broad nose bridge and nose tip, low-set ears with uplifted earlobes, bilateral post-axial polydactyly of the hands, and chronic ocular discharge due to bilateral nasolacrimal duct obstruction. Karyotype and FISH22q11 analyses were normal. Ophthalmologic investigation revealed bilateral coloboma of the papilla, and a small chorioretinal coloboma in the left eye. Renal and brain ultrasound were normal. Her parents and siblings do not show any feature of BOF syndrome. To our knowledge this is the first description of a patient with BOF syndrome with post-axial polydactyly.

P0041. Genetic polymorphisms, clinical risk factors and bone disease of preterms

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The analysis of risk factors for bone disease of very low birth weight (VLBW; <1500 g) infants is of special significance. In adults it has been shown that osteoporosis is associated with polymorphisms of vitamin-D-receptor (VDR), estrogen-receptor (ER), and collagen Iα1 (COLIA1) genes. No data are available whether genetic polymorphisms play a role in bone disease development in premature infants. We performed a pilot study to analyze the possible association between bone disease in VLBW infants and allelic polymorphisms of the candidate genes for osteoporosis, and we tried to identify fetal and maternal factors influencing bone turnover. Sixty-five VLBW infants were included. Twenty infants (30.8%) were diagnosed with bone disease based on positive radiological signs and high activity of bone formation (serum alkaline phosphatase, osteocalcin) and bone resorption (urinary excretion of calcium and pyridinolin crosslink) markers. Statistically significant correlation between $(Thymine\text{-}Adenine)_n$ repeat allelic variant of ER gene and bone disease was observed. Infants without

bone disorder carried more often a high number of repeats (TA>18) (OR: 0.17, 95%CI: 0.05-0.55). Low number of repeats (TA<19) was found more frequently in infants suffering from bone disease (OR: 6.00, 95%CI: 1.77-20.31). Furthermore, significant interaction (p=0.009) between VDR and COLIA1 genotypes was observed. In a logistic regression model using bone disease as dependent variable and controlling for different infant and maternal conditions, bone disorder of preterms significantly correlated with male gender (p=0.002), lower gestational age (p=0.015), homozygous allel variants of high number of $(TA)_n$ repeats (p=0.006) and interaction between VDR and COLIA1 genotype (p=0.009).

P0042. Blepharophimosis-ptosis-epicanthus inversus syndrome with additional anomalies due to an interstitial deletion of chromosome 3q

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We present a boy with blepharophimosis-ptosis-epicanthus inversus syndrome (BPES) and additional findings. Family history is non-contributory. Pregnancy was normal but culminated in delivery 9 days before term by caesearian section because of fetal hypoxia. Birth weight 2389 g (2.5%), length 47 cm (10%). Findings at birth included: blepharophimosis, ptosis, epicanthus inversus, anteverted nares, low-set posteriorly rotated ears, cleft palate, absent uvula, a right-sided inguinal hernia, a small umbilical hernia, short neck, adducted thumbs, gingival hypertrophy, second toe overiding hallux bilaterally and a partial lack of separation of penoscrotal skin. Feeding difficulties necessitated placement of a gastrostomy tube. At 3 months of age he is globally delayed with microcephaly.

Chromosome analysis by routine G-banding revealed an interstitial deletion of chromosome 3q (karyotype: 46,XY,del(3)(q24q25.3)). Micro-array CGH delineated a more centromeric *de novo* deletion; del(3)(q22.3q24). This region includes the *FOXL2* and *ATR* genes. Further investigations are planned to determine whether the deletion is maternal or paternal in origin.

Interstitial deletions of the long arm of chromosome 3 are uncommon and often give rise to BPES which maps to 3q23. Heterozygous mutations in, and deletions of, the *FOXL2* gene, which encodes a forkhead transcription factor, cause BPES. Deletions involving regions larger than 3q23 have been described in patients with BPES with microcephaly, mild mental retardation and growth delay. It has been suggested that these additional features result from the absence of a maternally inherited copy of the gene encoding ataxia-telangiectasia and Rad3-related protein (*ATR*).

P0043. Brachydactyly type C and angel-shaped phalangeal dysplasia: broadening the spectrum of CDMP1 mutations

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CDMP-1 is mapped on chromosome 20q11.2 and is closely related to the bone morphogenetic proteins. It is a cartilage specific member of the TGFß superfamily of secreted signalling molecules, expressed predominantly at sites of cartilage differentiation in developing limbs, where it may function as a signal for chondrogenesis, growth, and patterning of the developing vertebrate skeleton. There is a wide range of phenotypes associated with CDMP-1 mutations, from no discernable clinical and radiographical findings, to classic brachydactyly type C (BDC) with or without other skeletal manifestations, angel-shaped phalangeal dysplasia (ASPED), isolated short stature, vertebral abnormalities, and developmental dysplasia of the hip. In humans, homozygous and compound heterozygous CDMP-1 mutations cause acromesomelic chondrodysplasia (Grebe and Hunter-Thomson types), Du Pan syndrome or even BDC. Heterozygous mutations can lead to milder phenotypes, such as BDC, ASPED, brachydactyly type A2, mild shortening of 4th and 5th metacarpals or proximal symphalangism. In our study, CDMP1 molecular analysis has been performed in 12 families, comprising 3 with BDC, 3 with ASPED, 2 with Grebe syndrome, 1 isolated case with acromesomelic chondrodysplasia, and 3 families with either complex brachydactyly or acrodysostosis. So far 5 mutations have been identified (in the BDC or ASPED cases), including 3 which had not been previously reported. In these families, some individuals presented with non previously described features such as dental anomalies, renal malformations, or cardiac rythm anomalies. Although CDMP1 does not seem to be expressed in the kidney, it is expressed in the dental pulp and in the heart.

P0044. Fetal CNS malformations in a recurrence of Knobloch syndrome with splice mutation in COL18A1 gene

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Knobloch syndrome is a combination of posterior midline encephalocele, macular abnormalities, high grade myopia, vitreoretinal degeneration with retinal detachment and normal intelligence, suggesting alterations during early neuroectodermal morphogenesis. 28 cases have been reported insofar. It has been shown to be due to mutations in COL18A1 gene, mapped to 21q22.3. It leads to abnormal expression of collagen XVIII and endostatin. Endostatin is the non triple-helical C-terminal NC1 globular domain of collagen XVIII.

We followed a 3 year-old girl born to consanguineous Maghrebian parents with high grade myopia (-15d), perimacular pseudo-colobomatous lesions, septo-optic dysplasia (septal agenesis and optic nerve hypoplasia), symmetric frontal micropolygyria, occipital meningocele and subnormal psychomotor development. This girl harbors a splice site mutation in intron 36, which is predicted to alter the splicing of the RNA.

The next pregnancy was terminated at 17 weeks of gestation, because of the recurrence of the syndrome, discovered on presence of the posterior encephalocele. Pathological analysis showed a different pattern of brain malformation, with vermian agenesis and an hamartomatous lesion of the mesencephalic roof . These patients expand the range of CNS malformations observed with Knobloch syndrome and confirm the implication of endostatin in neuronal migration and CNS.

P0045. Branchio-Oculo-Facial syndrome in 2 unrelated patients - follow-up studies and new case report

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We presented clinical data of 2 children from different families with rare Branchio-Oculo-Facial syndrome (BOFS) and reviewed literature data for further delineation of phenotype.

Patient 1 (male) was born to a healthy 40-year old mother and 32-year old father. At infancy microcephaly, malformed ears, right-sided microphthalmia, cataract, ptoses, strabismus, nasolacrimal duct stenosis, broad nasal bridge, flattened tip, hypoplastic alae, high-arched palate, bilateral cleft lip and alveolar process, bilateral hemangiomatous cervical skin defects, brachydactyly, fifth fingers clinodactily were demonstrated. At follow-up examination (3-13 years old) he had growth, mental, speech delay, normal hearing, hypertrichosis, one absent incisor, dilatation of calicopelvis system (ultrasound). He attends a special education.

Patient 2 - a girl was the only child of young healthy parents, at birth bilateral cervical skin defects were documented. At first examination (1.5 years old) she showed normal growth and psychomotor development, high forehead, broad nasal bridge, flattened thick nasal tip, bilateral nasolacrimal duct stenosis, cyst of the upper lip, supra-auricular soft tissue mass on the left, cervical hemangiomatous skin defects partly healed, her distal phalanges of hands and foot was broad with pressed nails. Ultrasound scan detected two additional chords of heart and normal kidneys. Kariotype was normal of both children.

Our patients showed typical phenotype of BOFS, what was severe in boy and mild in girl. She had supra-auricular soft tissue mass and cyst of the upper lip, not previously described by BOFS. The wide expression of abnormalities requires careful examination of probands and parents to diagnose mild forms.

P0046. Optimal selection for *BRCA1* and *BRCA2* mutation testing using a combination of "easy to apply" probability models

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Purpose To establish a reliable and easy to apply risk assessment tool to select families for *BRCA* testing, using available probability models for *BRCA* mutation analysis.

Patients and Methods In 263 families, utility of the Frank (Myriad), Gilpin (FHAT) and Evans (Manchester) model to select 49 *BRCA* mutation positive families was analysed. For various cut off levels and combinations, the sensitivity and specificity were calculated and compared. The optimal combination was subsequently applied to two additional control groups.

Results Comparable sensitivity and specificity were obtained with the Gilpin and Evans models. They were shown to be complementary to the Frank model. To obtain an optimal sensitivity, 5 "individual criteria" were introduced that are specific for the selection of small or informationless families. The optimal selection criteria for the first serie is "Frank ≥ 16% or Gilpin ≥ 16 or one of five individual criteria". These criteria also proved valuable in the two other patient groups. In this combination, „Gilpin ≥ 16%" can be replaced by „Evans1 or 2 ≥ 10.

Conclusion Efficient selection of families for mutation testing of BRCA1 and BRCA2, can be improved by using a combination of risk assessment tools.

P0047. Association of ERa, ERbeta and AR genes with Breast cancer

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Genetic variation in genes involved in estrogen biosynthesis, metabolism and signal transduction have been suggested to play a role in breast cancer risk. To elucidate the possible role of genetic variation in the estrogen receptors α and β (*ER-α, ER-β*) and androgen receptor (*AR*) genes in breast cancer risk, the -1174(TA)$_n$, c.1092+3607(CA)$_n$ and c.172(CAG)$_n$ repeat polymorphisms of the three genes were studied. A case-control cohort of 79 women with breast cancer and 155 controls were used. No significant difference was observed in the frequency distribution of -1174(TA)$_{7\text{-}27}$ and c.1092+3607(CA)$_{10\text{-}26}$ in the *ER-α* and *ER- β* gene between patients and controls, while a significant difference was observed in the frequency distribution of repeat polymorphism c.172(CAG) $_{6\text{-}40}$ in the *AR* gene (p≤0.0001) with the mean number of CAG being higher in controls. A significant difference was observed in the repeat genotype distribution (SS, SL, LL) in the *ER-β* gene (p<0.0001) and for the *AR* gene (p≤0.0001). A significantly decreased odds ratio for breast cancer risk was observed in individuals having the SL genotype for *ER-β* gene compared to SS genotype (OR=0.013; 95%CI 0.004-0.041; p<0.0001) and LL genotype for *AR* gene (OR= 0.040; 95% CI 0.011-0.138; p<0.0001). The protective effect of SL genotypes for *ER-β* and LL for *AR* gene remained evident (p=0.004 and <0.0001) even after adjustment for various risk factors. In conclusion an association for breast cancer risk between short alleles for both c.1092+3607(CA)$_{13\text{-}27}$ and c.172(CAG)$_{8\text{-}34}$ repeat polymorphisms of the *ER- β* and *AR* was found in women of Greek descent.

P0048. Glucocorticoid receptor *Bcl*I variant in children with bronchial asthma.

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OBJECTIVE We investigated whether there were differences in allele and genotype frequencies of the *Bcl*I polymorphism in the glucocorticoid receptor gene among children with different levels of asthma severity and with different sex. The *Bcl*I polymorphism is a C/G substitution in intron 2, 646 nucleotides downstream from exon 2 (Van Rossum EFC et al., 2003).

SUBJECTS Our study group consisted of 485 children in age of 2-17 suffering from asthma, control group consisted of 151 healthy children in the same age (Table 1).

Methods The *Bcl*I polymorphism was detected by PCR-RFLP as described (Fleury I. et al., 2003). Allele and genotype frequencies were compared through Chi-square test.

RESULTS

Table 1 Genotype frequencies in asthma patients and controls

Genotypes	Study group (485)		Control group (151)	
	boys 396 (81,6%)	girls 89 (19,4%)	boys 78 (51,7%)	girls 73 (48,3%)
CC	40,4%	40,4%	32%	34,2%
CG	44,7%	43,9%	51,3%	52,1%
GG	14,9%	15,7%	16,7%	13,7%

Table 2 Genotype frequencies among children with different levels of asthma severity

Genotypes	levels of asthma severity					
	mild		moderate		severe	
	boys 89 (22,5%)	girls 12 (13,5%)	boys 216 (54,5%)	girls 45 (50,6%)	boys 91 (23%)	girls 32 (36%)
CC	41,6%	33,3%	39,4%	37,8%	41,8%	46,9%
CG	41,6%	58,3%	47,2%	48,9%	41,8%	31,3%
GG	16,9%	8,3%	13,4%	13,3%	16,5%	21,9%

CONCLUSION We not found differences in allele and genotype frequencies in asthma patients and controls and previously reported populations (Table 1). Allele and genotype frequencies were similar in groups of children with different levels of asthma severity, and with different sex (Table 2).

P0049. GENCOR: a national registry for patients and families suffering from a familial heart disease in the Netherlands

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Introduction: Developments in DNA-diagnostic techniques allow us to genotype an important amount of all patients with familial heart diseases (arrhythmias, cardiomyopathies etc.) and to identify family members with the same disease in early stages. Early treatment can prevent sudden cardiac death. However, data on long term outcome in unselected genotyped patients are scarce due to a lack of large registries. In 2005 a national internet-based registry for familial heart diseases in the Netherlands, named GENCOR, has been developed in collaboration with the Interuniversity Cardiology Institute of the Netherlands.

Objectives: GENCOR aims to assess the prevalence of familial heart diseases in patients and families in the Netherlands and to facilitate research to improve the quality of diagnostics and therapy in familial heart diseases.

Methods: Patients who visit the (cardio)genetic outpatient clinic are informed about GENCOR and consent is asked to store information about cardiac examinations, family history and DNA-diagnostics from all visits. Patients' data are entered in GENCOR by the cardiologist or

clinical geneticist in attendance. Additional information can be stored for scientific research.
Results: The registry has been tested in two university hospitals which resulted in the inclusion of more than 200 patients. In 2006 all university hospitals will start using GENCOR. Two research projects have already started using GENCOR.
Conclusions: GENCOR is already a success, regarding the number of included patients and the related research projects in a limited period of time. GENCOR provides easy internet-based access for authorized scientists throughout the country.

P0050. Haplo-insufficiency of the Cat eye critical region has no clinical relevance

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The presence of four highly homologous sequences, known as low copy repeats (LCRs), predisposes for unequal recombination within the 22q11 region. This can lead to genomic imbalances associated with several known genetic disorders. We report a developmentally delayed patient with congenital malformations carrying three different rearrangements on both chromosome 22 homologues, including a previously unidentified deletion of the 22q11.1 region. Although four copies of the region are known to cause the Cat eye syndrome, we postulate that a deletion of this region has no clinical relevance, as five healthy family members also carried this deletion. In addition, the patient had a duplication of the DiGeorge critical region (22q11.2) and a deletion of chromosome band 22q12.1. As several clinical features, present in our patient, did not fit in the spectrum of the duplication 22q11.2 syndrome, it is argued that an interstitial deletion of chromosome band 22q12.1 might be related to the congenital malformations seen in the proband.
This study highlights the value of using different genomic approaches (MAPH, MLPA, tiling-path-array and FISH) to unravel chromosomal alterations in order to study their phenotypic impact.

P0051. Congenital Central Hypoventilation Syndrome: Clinical and molecular review of a UK cohort of 22 cases with PHOX2B mutations.

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Congenital central hypoventilation syndrome (CCHS; OMIM: 209880) is a rare, life-threatening disorder, characterised by an impaired response to hypercapnia and hypoxia, resulting in hypoventilation. Other autonomic nervous system anomalies such as Hirschsprung disease and neuroblastoma, may also be present.
Heterozygous mutations in the paired-like homeobox gene, PHOX2B (OMIM:603851) occur in up to 98.5% of CCHS patients. The most common mutations are expansions of a 20-residue polyalanine tract by +5 to +13 alanines. Other mutations described include nonsense mutations and frameshift deletions/insertions.
Bristol has provided a UKGTN molecular testing service for PHOX2B mutations since February 2005 and has identified mutations in 22/33 probands, including 22/25 with a clinical diagnosis of CCHS. Three of the mutations were found in neonates, permitting rapid confirmation of the diagnosis. Two of the three patients with clinical CCHS but no PHOX2B mutation had multiple hypothalamic abnormalities including disorders of thermoregulation as well as sleep related hypoventilation. Mutations found in this cohort were all polyalanine expansions (+5 to +12 residues), except for one 38bp frameshift deletion beginning within the first codon of the polyalanine tract.
Molecular investigations of parents in 14/22 cases revealed two full mutation carriers (one known to be symptomatic) and two asymptomatic mosaic cases, suggesting a higher frequency of mosaic cases than was previously reported.

P0052. Association between Alpha-1-Antitrypsin mutations and sevierity of CF

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Cystic Fibrosis (CF) transmembrane conductance regulator (CFTR) genotype does not explain the heterogeneity observed in CF pulmonary disease severity. Modifier genes are implicated for this heterogeneity. Alpha-1-antitrypsin (Alpha-1-AT) is one of the few antiprotease capable of inactivating neutrophil elastase. We investigated whether Alpha-1-AT alleles (Z, S deficient alleles and The 3' $G_{1237} \rightarrow$ A mutation) is associated with increased disease severity and Alpha-1-AT acute phase response during pulmonary exacerbations.
70 CF patients were genotyped for the S and Z, Mutation and $G_{1237} \rightarrow$A polymorphism of the Alpha-1-AT gene using PCR-RFLP Method.
200 Control individuals were also screened for G→A mutation in order to find the frequency disease.of this polymorphism in Iranian population.
Our result suggest no correlation between allelic alteration in Alpha-1-AT gene and CF.

P0053. CFTR gene mutations in Iranian Cystic Fibrosis patients

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Numerous mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been found to impair CFTR activity to different extent, causing CF. This disorder exhibits considerable allelic heterogeneity in different populations and ethnic groups. Due to very high heterogenousity of Iranian population identification of mutations specific to the Iranian population would be helpful in designing an appropriate CF molecular diagnosis.
80 blood samples from unrelated CF families were collected from different provinces of Iran. DNA samples were screened for the most common mutations including delF508, G542X, W1282X, and N1303K using ARMS-PCR. Exons 4, 7, 9, 10, 11, 13, 20, and 21 were screened using SSCP-Sequencing.
DelF508 mutation covered only 18% of the mutated alleles, followed by W1282X(11%), G542X(7%) and N1303K(2.5%) respectively. SSCP-Sequencing also revealed the occurrence of some rare mutations including R117H, R347H, A120T, S549R, 1677delTA and 2183AA>G. Moreover M470V mutation was found in high number of Iranian CF patients.

P0054. Molecular analysis of Charcot-Marie-Tooth in Spanish patients negative for the *PMP22* duplication

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Charcot-Marie-Tooth (CMT) disease is the most common inherited neuropathy and more than 19 genes have been involved to date. This genetic heterogeneity, together with the fact that sometimes the same gene accounts for different CMT subtypes and/or inheritance patterns complicate diagnostic decisions. Genetic epidemiology of CMT in a given population is essential in order to establish a rational protocol for molecular analysis. We investigated a group of 47 Spanish patients (mostly from the Galician region) with a clinical diagnosis of probable CMT and who had tested negative for the duplication of a chromosomal segment containing *PMP22*, the most frequent molecular alteration in demyelinating CMT. Systematic sequencing of the coding region and exon-intron junctions of *PMP22*, *MPZ*, *GDAP1*

and *LITAF* was performed. We found two patients carrying previously described mutations in *MPZ*: the first had a G to A substitution in exon 3 (R98H) and the second, a 486delC in exon 4 causing a frameshift mutation (M172fs262). Additionally, we identified two novel coding sequence alterations: i) one patient had a G to C substitution in the first coding exon of *PMP22*, predicting a leucine to phenylalanine change in the protein (L5F) and ii) two apparently unrelated patients exhibited a heterozygous GAA deletion in exon 5 resulting in the loss of an arginine (R224del) in the GDAP1 protein. Other five nucleotide substitutions not contained in dbSNP were found in intronic regions. We describe the phenotype of the patients carrying these sequence changes and discuss their possible role in the development of CMT.

P0055. CHARGE syndrome -mutational analysis of CHD7 gene in Finnish patients

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CHARGE (MIM # 214800) is a frequently diagnosed syndrome in infants (1 in 8500 births) and has been named after a pattern of congenital anomalies: coloboma, heart malformations, choanal atresia, retardation of growth and/or development, genital abnormalities and ear anomalies. Recently, heterozygotic mutations in *CHD*7 gene located in 8q12 were proven to cause ~60% of clinically diagnosed cases. Most of the mutations are intragenic, but in few cases microdeletions encompassing the whole *CHD*7 gene have been detected. In individual cases mutations have also been detected in the *SEMA*3E gene and in the chromosomal area 22q11.2, thus indicating locus heterogeneity.
The purpose of this study is to set up molecular diagnostic test for this syndrome, clarify mutational spectrum in Finnish patients and give tools for clinicians to improve clinical diagnostics. To achieve this, samples of clinically diagnosed patients and parental samples have been collected from Clinical Genetics Units of the five University Hospitals in Finland. The coding area and intron-exon boundaries of *CHD*7 gene are analysed by direct sequencing to detect intragenic mutations, and samples without intragenic mutations are screened with quantitative real-time PCR to detect possible microdeletions in 8q12 area. The results are currently being analysed and will be reported. Even though Finnish population has a very unique genetic background our hypothesis is that in case of CHARGE the mutation spectrum will turn out to be similar to other European populations.

P0056. A CHARGE-like syndrome in two sibs

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CHARGE syndrome (OMIM #214800) is a well known MCA syndrome that usually occurs sporadically. Recently, *CHD7* gene (Chromodomain Helicase DNA binding 7 gene) has been shown to be mutated in roughly 60% of the cases. The pathogenic mechanism seems to be haploinsufficiency. Furthermore, a mutation of *SEMA3E* gene was reported in a single case.
We report two brothers with an association of abnormalities compatible with CHARGE syndrome. Both have vestibular dysfunction associated with hypoplastic semicircular canals, mental retardation and facial dysmorphism with dysplastic ears. None has choanal atresia and only patient 2 presents with coloboma. Patient 1 has multiple congenital abnormalities: bilateral cleft lip and palate, esophageal atresia with tracheo-oesophageal fistula, complex heart defect and vertebral abnormalities. Patient 2 has no mediastinal malformation, but is epileptic. The mother has some signs that could be compatible with a mild CHARGE syndrome.
Familial cases of CHARGE syndrome are rare. It is now established that CHARGE syndrome is an autosomal dominant disorder with a variable penetrance. Proven recurrence of *CHD7* gene mutation in two sibs has already been reported in relation with maternal germinal mosaicism, and somatic mosaicism of *CHD7* mutation has been demonstrated in one mother. Nevertheless, a recessive CHARGE-like syndrome remains - theoretically - a hypothesis.
Unfortunately, as of this abstract submission, results of *CHD7* gene study in this family are not yet available. Whatever these results may be, this familial report shows the familial variability of the presentation of CHARGE syndrome.

P0057. Deletion within chromosome 22q11 in two subjects with CHARGE syndrome

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The acronym CHARGE, coined by Pagon, refers to a non-random cluster of characteristic associated anomalies, first described by Hall, including ocular coloboma, **c**hoanal atresia, hearing loss, cardiovascular malformations, urogenital anomalies and retarded growth and development, ear abnormalities and/or hearing loss. Hypogonadotropic hypogonadism and abnormal olfactory bulb development were recently included in the phenotypic spectrum. Arhinencephaly and semicircular canal agenesis were two constant features in all observed fetuses with CHARGE syndrome and CHD7 mutations. CHARGE syndrome, initially referred to as an *association*, is now a well-established multiple-malformation *syndrome* with distinctive consensus diagnostic criteria. Recent studies have showed that the syndrome is caused in about 60% of cases by CHD7 gene mutations, a chromodomain helicase DNA-binding protein gene, on chromosome 8q12.1. Because a locus on chromosome 22q11 is deleted in most individuals with DiGeorge, Shprintzen syndromes or other isolated or syndromic heart abnormalities, we have searched for deletions in eight children with CHARGE syndrome associated to outflow-tract heart defects. Deletions within chromosome 22q11 were found in two subjects. CHD7 mutations and/or 22q11 deletions may thus be regarded as contributing factors to genetic heterogeneity of CHARGE syndrome.

P0058. CHARGE-like phenotype and neurofibromatosis type 2 due to an intersitital deletion of chromosome 22q

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CHARGE is an acronym for **C**oloboma, **H**eart defects, **A**tresia choanae, **R**etardation of growth and/or development, **G**enital defects, **E**ar anomalies and/or deafness. Mutations in the *CHD7* gene on chromosome 8q cause CHARGE syndrome.
CHARGE syndrome was diagnosed in a boy with choanal atresia, an ASD, preauricular tags, an undescended testes, right-sided microphthalmia, left-sided corneal opacification, scoliosis and a bladder diverticulum. Transient swallowing difficulties resolved after infancy. His hearing and sight were severely congenitally impaired. He did not have ocular colobomas, documented congenital cranial nerve dysfunction or known abnormalities of the semi-circular canals.
His karyotype on routine G-banding was 46,XY and there was no deletion of 22q11.2 with FISH technique.
He acquired pigmented nodular skin lesions in childhood. During his late teens he became withdrawn and passive. His hearing and balance deteriorated and he stopped walking. A gastrostomy was inserted because he stopped eating. When he died at age 22, autopsy revealed the cause of death to be bilateral vestibular schwannomas i.e. neurofibromatosis type 2.
Post mortem microarray CGH on a banked blood sample revealed a 6 Mb *de novo* deletion approximately 6 Mb distal to the DiGeorge syndrome locus encompassing about 100 genes including the *NF2* gene. Revised karyotype: 46,XY .rev ish dim(22)(q11.23q12.2). Mutation analysis revealed no abnormality of the *CHD7* gene.
In conclusion, neurological deterioration is not a feature of CHARGE syndrome. Progressive neurological signs and symptoms in a person diagnosed with CHARGE syndrome should be investigated and should prompt diagnostic re-evaluation.

P0059. The presence of genetic disorders associated chest wall malformations

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Background: Although the pectus excavatum is the most common malformation of the anterior wall of a child's thorax, today the causes that determine the appearance of this malformation are not known. There is increasing evidence nowadays that genetics plays an important role in the appearance of chest wall malformations.
Objectives: The purpose of this study was to identify the cases that have associated with the chest wall malformation a genetic condition that might have a role in the appearence of the malforation in the first place .
Material and Method: The present is a retrospective study that analizes a number of 121 medical records of pacients affected by chest wall malformations such as pectus excavatum , pectus carinatum treated at the Childrens Hospital Louis Turcanu ,Department of Pediatric Surgery in Timisoara,during 1986-2003 .
Results: In the analyzed group of subjects 5 patients presented Poland syndrome ,1 had Marfan syndrome, 1 presented Turner syndrome and 1 had associated with the chest wall malformation syndactily of the hands.
The male/female ratio was 2.1/1 in favor of male patients. The average age range was between 5-17 years,80% patients being teenagers.
Many patients with postoperative relapses were those that had associated with their condition a genetic disorder.
Conclusions: With regards to the conclusions of this study, we noticed the importance of genetical disorders that undoubtedly complicate the management of the chest wall malformations.
It is equally important for these patients to get a genetics specialist counselling to improve the outcome of their condition.

P0060. Chiari type I malformation in six unrelated patients affected with Fabry disease

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Fabry disease (FD, OMIM 301500) is an X-linked lysosomal storage disorder caused by one of numerous mutations of the *GLA* gene encoding lysosomal enzyme α-galactosidase A. The inherent metabolic defect leads to intralysosomal accumulation of neutral glycosphingolipids. The disease is associated with ischaemic complications involving kidneys, heart and brain, severe multi-organ dysfunction, and premature death. Among neurological symptoms, strokes and transient ischaemic attacks (TIA) have been reported. A 30-year-old male patient, with FD, was referred to us for evaluation of a sudden episode of dizziness, with disequilibrium, and diplopia, in agreement with the diagnosis of a TIA. Head magnetic resonance imaging (MRI) showed no cerebrovascular involvement but revealed the presence of Chiari type I malformation (CMI), a pathological *continuum* of hindbrain malformations defined by downward herniation of the cerebellar tonsils of more than 5 mm below the *foramen magnum*. We subsequently performed head MRI in a cohort of 44 consecutive hemizygous male patients and 10 heterozygous females affected with FD, and identified 4 additional cases (one male and three female) and one case with borderline criteria of CMI. To the best of our knowledge, this is the first time that CMI was found in association with Fabry disease. Whether this is coincidental or whether there is an increased occurrence of CMI in FD is yet unknown but our data suggest that CMI should be ruled out in all patients with FD.

P0061. Two different chromosomal anomalies in siblings: Klinefelter syndrome and del(18)(q21)

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There are very few case reports about different chromosome rearrangements in siblings born to cytogenetically normal parents. We would like to present a family with two different chromosome rearrangements in two siblings. The proband, a girl, was investigated shortly after birth because of multiple congenital malformations (cleft lip and cleft palate, bilateral talipes, short fourth fingers, epicanthi and narrow palpebral fissures). Subsequently chronic bilateral neuritis cochlearis with a partial hearing loss was diagnosed. Cytogenetic analysis performed from peripheral blood lymphocytes using standard G banding technique resulted in the karyotype 46, XX, del(18)(q21). Proband's elder brother showed some phenotypical abnormalities such as tall stature, long slim arms, scoliosis. His karyotype was 47, XXY (Klinefelter syndrome). The karyotypes of both parents were normal. Mother's age at birth of the son and the daughter was 22 and 29 years, respectively.
The phenomenon of different chromosomal rearrangements in siblings born to cytogenetically normal parents implies the necessity to investigate the genetic, environmental or complex causes of abnormal gametogenesis. One possible explanation could be a not yet determined error in DNA repair.

P0062. Chromosome 7 duplication in a young girl with congenital heart disease, unusual behaviours and low IQ level: clinical and cytogenetical characterisation

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We report the case of a 15 years-old girl, who was found to carry a chromosome 7 duplication by karyotype analysis. The proband was referred for genetic evalution because of behavioural disturbance and abnormal anxiety.
Clinical examination found congenital heart disease, unusual behaviours, partial simian line in both palms, movement difficulties and low IQ (60 percentage) level. The most frequent abnormality of chromosome 7 is as trisomy or as polysomy, which is present in 65-90% of reported cases. A partial trisomy chromosome such as in our patient is a rare event and represents only 13% of all trisomies. The oncogene EGFR that is located on chromosome 7p12 is the most frequently amplified gene in astrocytic tumors of glioblastoma multiform types.
Conventional cytogenetic analysis identified an abnormal chromosome 7 for the proband and her mother, whereas other autosome chromosomes were normal. All methaphases showed 46,XX,dup(7)(p^{ter})mat in her final karyotype. The segmental duplication of chromosome 7 is discussed based on the present results**.** The current proband showed no signs of the Williams syndrome. As is found in patients with deletion of the long arm of chromosome 7. In this study, we analyzed the relationships between a segmental duplication of chromosome 7 and behavioural disturbance, congestive heart disease and abnormal anxiety in a case. Our results suggest that specific genes at $7p^{ter}$ are exquisitely sensitive to dosage alterations that can influence human low IQ and special behavioural disturbance capabilities.

P0063. A novel missense mutation in CIAS1 encoding the pyrin-like protein, cryopyrin, causes familial cold autoinflammatory syndrome in a family of Ethiopian origin

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Familial cold autoinflammatory syndrome (FCAS MIM 120100) is a rare autosomal dominant condition characterized by unremitting attacks of cold-induced urticaria, often accompanied by other systemic manifestations. The disorder was previously shown to be caused by mutations in CIAS1, encoding a pyrin-like protein also involved in the pathogenesis of Muckle-Wells syndrome (MWS MIM 191900), and CINCA syndrome (Chronic Infantile Neurological Cutaneous and Articular syndrome, MIM 607115). In the present study, we assessed a two-generation family of Jewish Ethiopian origin, including 3 members affected with FCAS. Using direct sequencing, we identified a novel CIAS1 mutation, F525C. The mutation was shown to affect a highly conserved residue of the protein and to segregate with the disease throughout the extended family. Our results add to the expanding

spectrum of mutations in CIAS1 and provide evidence for striking phenotypic heterogeneity in inherited autoinflammatory syndromes .

P0064. The first case of Chronic Infantile Neurological Cutaneous and Arthropathy (CINCA) syndrome in Lithuania

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Background: Chronic Infantile Neurological Cutaneous and Arthropathy (CINCA) syndrome, also called Neonatal Onset Multisystem Inflammatory Disease (NOMID) is a rare congenital inflammatory disorder characterized by the triad of permanent cutaneous rash from birth, central nervous system involvement and joint manifestations with recurrent fever and inflammation. About 100 cases are reported worldwide although this syndrome is clearly underdiagnosed. Mutations in CIAS1 gene are reported up to 60% of CINCA cases, but exact underlying pathogenic mechanisms are still unclear.
Case report: We report the first sporadic case of CINCA syndrome in Lithuania diagnosed in a 8 year old boy. Diagnostic sequence ranging from allergic dermatitis, juvenile idiopathic arthritis and hypopituitarism is presented and clinical disease course till 16 years is described. Characteristic findings of the syndrome phenotype were observed as follows: alternate non-itching urticaria present from birth; chronic meningitis reflected by occasional headache and vomiting, atrophy of the optic nerve, optic disc edema and recurrent febrile attacks; limited mobility, progressively deforming symmetrical arthropathy of elbows and knees with typical metaphyseal and epiphyseal radiological changes, abnormal ossification, patellar overgrowth, clubbing of fingers and small stature. Skull anomalies including macrocephaly, hydrocephaly, frontal bossing, saddleback nose and late closure of fontanelles were obvious. Laboratory analyses documented a non-specific inflammatory response. A progressive visual defects and perceptive deafness occurred with increasing age. Some symptomatic peculiarities are also discussed.
Conclusion: Despite characteristic features of CINCA syndrome only the long term clinical observation helps to differentiate it from other types of early onset chronic arthritis with systemic manifestations.

P0065. Congenital lobar emphysema: a report of ten cases from one genealogy

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Congenital lobar emphysema (CLE) [MIM130710] is an autosomal dominant lung disease with hyperinflated lobe of the lung, compressed normal lung tissue, bronchial cartilage hypoplasia. Here we report a family with 10 cases of CLE. The first patient was a 40 years old man, who had symptoms of chronic obstructive lung disease, lobar emphysema, history of two events of sporadic pneumothorax. KT showed left upper lobe emphysema with compression of other part of the lung. Thoracotomy and left upper lobectomy were performed for decompression of healthy part of the lung. Biochemical analysis for quantitative alpha-1 antytripsin level in serum was at lower range of normal limits. Genealogy showed 9 more cases of CLE. Affected members were almost in all generations. In all cases disease manifested after puberty. Both of the proband's children (son and daughter) also had lobar emphysema. Other affected members were a cousin, two sisters, a nephew, two nieces and grandmother. It is interesting that proband's father (son of affected grandmother) was healthy. We think, that incomplete penetrance is the reason why patients' father is healthy. We represent the most numerous family with late onset of CLE in Lithuania.

P0066. Associated malformations in cases of oral clefts

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Infants with oral clefts(OC) often have other associated congenital anomalies.The reported incidence and the types of associated malformations vary between different studies.The aim of this study was to assess the prevalence of associated malformations in a geographically well defined population.
Associated malformations in infants with OCs were collected between 1979 and 2003 in 334,262 consecutive births in the area covered by the registry of congenital anomalies of Northeastern France.
The results showed that 35,9 % out of the 651cleft infants born during this period had associated malformations.Associated malformations were more frequent in infants who had cleft palate(47,9 %) than in infants with cleft lip and palate(34,9 %) or with cleft lip only(14,4 %).
Malformations in the central nervous system and in the skeletal system were the most common other anomalies, followed by malformations in the urogenital and cardiovascular systems.
Weight, length, and head circumference of children with OCs and multiple associated malformations were lower than in controls
Prenatal diagnosis was rarely done by fetal ultrasonographic examination in isolated clefts. However, even in multiple associated malformations, prenatal diagnosis by fetal ultrasonographic examination had a low sensitivity,43.9%.
In conclusion the overall prevalence of malformations, which was one in more than three infants, emphasizes the need for a thorough investigation of infants with clefts. A routine screening for other malformations especially skeletal, central nervous system, and cardiac defects may need to be considered in infants with clefts, and genetic counseling seems warranted in most of these complicated cases.

P0067. Symptomatic Charcot-Marie-Tooth. A pair of concordant monozygotic twins

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A pair of monozygotic twin brothers was referred due to hereditary peripheral neuropathy. The clinic and neurophysiology was compatible with Charcot-Marie-Tooth disease with late onset. Molecular genetic analysis excluded mutations in PMP22, connexin32 and MPZ. The twins were employed in PVC production and developed symptoms after 14 years of massive exposure. We conclude that the monozygotic twin pair have symptomatic CMT, i.e. a phenocopy, since it is primarily caused by environmental and not by genetic factors. Phenocopies occur, but it is extraordinary that the two patients in a family do have a symptomatic rather than the hereditary form of CMT. This illustrates the importance of an occupational history even in the molecular genetic era.

P0068. Frequency of 17p11.2 duplication/deletion in a group of Portuguese patients with Charcot-Marie-Tooth type 1 (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP)

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CMT type 1 and HNPP are autosomal dominant sensory motor peripheral neuropathies. The most frequent mutations are a reciprocal duplication and deletion of one copy of the gene encoding peripheral myelin protein 22 (*PMP22*), at the *CMT1A/HNPP locus* (chromosome 17p11.2). CMT is characterized by slowly progressive weakness and atrophy of distal muscles in the feet and/or hands, often associated with diminished deep tendon reflexes. Nerve conduction velocities, when carefully performed, are frequently decreased (10-30m/s). Molecular testing, performed since 2001, detects above 95% of patients with CMT1A and approximately 100% of the HNPP cases.
To ascertain the frequency of duplication/deletion of *PMP22*, we analyzed 84 Portuguese patients referred for molecular diagnosis of CMT1A/HNPP, for seven highly polymorphic CA repeats (D17S921, D17S955, D17S839, D17S122, D17S1357, D17S1358, D17S1356), one tetrametric (4A) and one pentametric repeat (9A), spanning the 1.5 Mb duplicated in CMT1A and deleted in HNPP. The duplication occurred in 18 patients and the deletion only in one. Among all markers, 9A was the most informative, detecting 14/18 (77.7%) of all CMT1A cases, while D17S122 detected 10/18 (55.5%).

Confirmation of CMT1 (detection of the *PMP22* duplication) was achieved only in a small number of the cases (21%). This could be due to an incomplete clinical characterization: if we considered only the cases with known family history or with suggestive electrophysiological signs, the frequency of CMT1A molecularly confirmed cases would rise to about 45% (14/31). A more detailed clinical characterization is essential, including EMG studies and NCV for a better diagnosis of CMT.

P0069. De novo homozygous tandem duplication in a Spanish patient affected with CMT1A

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Charcot-Marie-Tooth disease type 1A is an autosomal dominant demyelinating neuropathy where PMP22 gene is implicated. Two mechanisms are responsible for CMT1A: **1)** Dosage effect of PMP22; **2)** Point mutations of the gene.

The former mechanism is due to a tandem duplication of a 1.5Mb region originated from an unequal crossover during meiosis and it is present in over 70% of patients. Sporadic autosomal dominant CMT type 1 cases cannot clinically be differentiated from recessive CMT.

Diagnosis in our laboratory is performed with 11 STR markers that are located inside the microduplication as well as using the MLPA system.

We report a sporadic case of a woman affected with CMT1A that shows a *de novo* homozygous tandem duplication. DNA from parents was required to construct the haplotypes and a false paternity was ruled out with informative markers located on chromosomes 13, 18, 21 and X.

The duplication was originated from the same paternal haplotype and was also observed in a foetus from this affected patient.

We propose that an unequeal crossover occurred in a mitotic division during an early embryonic stage, prior to the formation of the three embryonic layers. The presence of a disease phenotype points obviously to the ectoderm; the presence of the actual duplication in our molecular analysis of blood tissue, points to mesoderm; and the fact that the patient transmitted the duplication to the next generation points also to the endoderm.

P0070. Novel mutation of the myelin P0 gene (*MPZ*) in a Portuguese family with CMT1B disease

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Charcot-Marie-Tooth disease type 1B (CMT1B) is an autosomal dominant peripheral neuropathy associated with mutations of the major peripheral myelin protein (Po) gene (*MPZ*). The Po protein functions as a homophilic adhesion molecule in myelin compaction. As other CMT1 types, CMT1B is a demyelinating peripheral neuropathy characterized by distal muscle weakness and atrophy, sensory loss, and slow nerve conduction velocity. It is usually slowly progressive and often associated with pes cavus foot deformity and bilateral foot drop. The range of the phenotypes associated with these neuropathies is very wide, and correlations between the phenotypes and the individual mutations are useful.

Here we report a portuguese family with CMT1B associated with a novel Po point mutation. Four clinical affected members of three consecutive generations had a heterozygous mutation (278G>C) in exon 3, leading to a Gly93Ala substitution. In healthy family members no Gly93Ala was found. Gly93 is conserved among many other species suggesting that it is important in the formation of an extracellular Ig domain. Ikegami *et al*. (1) reported a family with CMT1B associated with a Po point mutation in the same position of exon 3 (278G>A, Gly93Glu).

The age of onset of symptoms varied from 11 to 51 years, suggesting that in addition to the type and intragenic location of the mutation, other mechanisms acting at the level of *MPZ* gene expression, mRNA stability and posttranslational protein modification may have an important effect on the ultimate clinical phenotype.

(1) Ikegami T. *et al*. Am. J. Med. Genet. 71:246-248(1997).

P0071. Arg94Gln mutation in the *MFN2* gene is most frequent in the patient with Charcot-Marie-Tooth type 2 disease from Russia.

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Charcot-Marie-Tooth neuropathy (CMT) is a genetically heterogeneous group of hereditary nervous - muscular diseases with a similar clinical picture. There are two big groups of Charcot-Marie-Tooth disease: myelinopathy (CMT1) and axonopathy (CMT2). Both consists of further subtypes. CMT2A is the most frequent type among axonopathies, it is caused by mutation in the gene of mitochondrial GTPase Mitofusin 2 (*MFN2,* 1p36.2).

We have investigated 59 families with a clinical diagnosis of CMT2 and 28 families with CMT unknown type, in total 87 unrelated families. Mutations have been found in 10 families (all of them have diagnosis CMT2). The most frequent mutation is Arg94Gln. We have found it in five unrelated families. We have analyzed the cause of the high frequency for this mutation by haplotype analysis using five polymorphic markers and seven intragenic SNPs. We determined that this mutation had independent origin in various families. In two other families a mutation Arg94Trp was found in the same codon. These dates lead us to conclude that codon 94 is «hot spot» for mutation in the *MFN2* gene.

P0072. High Prevalence of Cohen Syndrome amongst Irish Travellers

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Cohen syndrome is a rare autosomal recessive disorder, prevalent in the Finnish and Jewish populations. We report five cases of Cohen syndrome amongst three Irish Traveller families from Galway. We are aware of a sixth case in a UK based Irish Traveller child. All six have an identical homozygote mutation c.4471G>T mutation in the COH 1 gene. Using Traveller population data, from the 2002 census, we estimate a minimum prevalence of Cohen syndrome of 8 per 1000 amongst Irish Traveller children in the County of Galway.

There are >30 other autosomal recessive conditions known to occur in this population. The prevalence of autosomal recessive disorders is unknown but a recent study in County Galway reported that 2 % of Traveller children have Hurler syndrome, 2% have Galactosemia, 1% have autosomal recessive OI and 3% are deaf. In addition, a further 5% had some form of disability and 28% of Traveller parents have suffered the death of a child.

A delay in diagnosis occurred as many of the features were only apparent in later childhood and, in the context of this community, follow up is difficult as families move.

The UK based case and our cases 4&5, who are unrelated to case 1,2 & 3, suggest that this disorder occurs throughout this community and is not isolated to one clan. Molecular confirmation in this population is easy because the mutation is known. We recommend considering this diagnosis in any child from this population who presents with developmental delay and microcephaly.

P0073. Report of 10 new patients with a heterozygous mutation in the COL11A1 gene and review of genotype-phenotype correlations in type XI collagenopathies.

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A series of 35 unrelated patients with variable features of either

Stickler or Marshall syndrome and normal COL2A1 screening were investigated for mutations in the COL11A1 gene. Heterozygous COL11A1 mutations were found in 10 individuals. In 8 cases a splice site alteration (involving introns 44, 47, 50, 53, 55) was present with one (c.3816+1G>A) being recurrent in two patients. One patient had an in-frame deletion of 18 nucleotides in exon 36 and in another patient a missense mutation (Gly1471Asp) was observed. In 6/10 patients the phenotype was classified as Marshall syndrome because of the early-onset severe hearing loss and the severe midfacial retraction with short nose and protruding eyes. Ectodermal abnormalities were absent in all patients but myopia and retinal detachment observed in some of them. One patient even had a type 1 vitreous anomaly which has been considered to be specific for Stickler syndrome type 1 (caused by mutations in COL2A1). In this group of 6 patients, 5 had a splice mutation and 1 had the 18 nt deletion in exon 36. The phenotype in the remaining 4/10 patients was similar to Stickler syndrome type 1. The midface hypoplasia was less pronounced and only one patient presented with early-onset sensorineural hearing loss. We conclude that (in contrast to previous reports) splice mutations in COL11A1 can result in either a Marshall phenotype or a Stickler phenotype. We also show that the vitreous phenotype is not always reliable in predicting the molecular defect in patients with the Stickler-Marshall phenotypic spectrum.

P0074. An audit of a mutation screening service for the 21-hydroxylase gene

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Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive genetic disorders, which result in a deficiency of enzymes in the cortisol synthesis pathway. The majority (>90%) of CAH is caused by 21-hydroxylase enzyme deficiency (21OH).

In response to concerns about the management of CAH patients in the UK an audit of CAH referrals to the laboratory was conducted. A total of 495 CAH families referred for molecular testing of the *CYP21* gene between January 2000 and June 2005 were examined. The audit highlighted a requirement for further information on the complex genetics of this disease and indicated that current population carrier risks may need reviewing [N.Gregersen *et al*. 2006. In preparation].

Another area the audit highlighted was the correlation between genotype and phenotype. In patients where two mutated alleles had been identified we compared the expected mutation effect to the phenotype of the patient. The phenotype was as predicted from the identified genotype in 80.7% of cases. It was also noted that in 31% of patients only one mutation had been identified. To increase our detection rate we have developed a rapid test, using pyro-sequencing, for another relatively common mutation in CYP21, the exon 6 cluster mutation (g.1380T>A, g.1383T>A). To date we have identified 12% (5/42) of patients, where only one mutation had previously been identified, as positive for the exon 6 cluster mutation. This new test avoids the need for sequencing the majority of the CYP21 gene, a time consuming and expensive test.

P0075. Warburg Micro Syndrome in a Turkish Boy

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We report here a 4-year-old Turkish boy with Warburg Micro syndrome (WMS) who was born to consanguine parents and was referred due to psychomotor retardation and cataract. He had similar physical features and findings with patients reported previously with WMS like; microcephaly, deep-set eyes, prominent ears and nasal root, micrognathia, hypertrichosis and truncal hypotonia,spastic diplegia,joint contractures, hypogenitalism and hypoplasia of corpus callosum. Sequence analysis of exon 8 of the RAB3GAP gene has confirmed the presence of a splice donor mutation (748+1G>A) in the homozygous state. Skin hyperextensibility and joint hypermobility in the affected child have not been reported in WMS cases to date.

The purpose of this report is to compare the symptoms and features of the case with previously reported cases with Warburg Micro syndrome as well as with the other syndromes including common findings and features.

P0076. Congenital malformation - a risk factor for infectious pathology

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Introduction: Congenital malformations represent a fundamental pathology problem correlated to incidence, etiology, pathology and medical and social implications.

Purpose: This study wants to establish the correlation between congenital malformations of the newborns and the infections pathology they developed.

Material and method: Clinical examinations and laboratory data have been used for this study.

Results: Of a total number 2610 hospitalized new born 6,89% had congenital malformations, 3,73% male and 3,54% female. The types of malformations are cardiovascular 2,68%, gastrointestinal 0,28%, the urinary tract 0,38%, central nervous system 0,38% and complexes malformations 0,09%.

The infectious pathology of the new born is he following: skin and mucous 4,02%, respiratory infection 3,64%, bacteremia 1,15% and urinary infections 0,86%.

Of a total number of positive cultures we found staphylococcus aureus 65,65%, e. colli 18,32%, enterobacter 6,87%, klebsiella 3,05%, staphylococcus R 3,05%, pseudomonas aeruginosa 2,29%, staphylococcus epidermidis 0,76%.

Maternal infections circumstances: the premature rupture of the amniotic membranes 22,22%, green amniotic fluid 30,55%, maternal infections 19,44%.

Conclusions: 1. because of the immaturity of the imunitary system at the new borns with congenital malformations, the infections pathology is more frequent; 2. the most frequent bacterial agent are staphylococcus aureus and e. colli.

P0077. Congenital pulmonary lymphangiectasis in two female half-siblings.

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We report on two female half-siblings with congenital pulmonary lymphangiectasis (CPL). These children were born to a healthy mother with a negative family history regarding congenital anomalies. Both pregnancies had been achieved by *in vitro* fertilization using different anonymous, unrelated sperm donors. The first child was prenatally diagnosed with hydrops fetalis and bilateral hydrothorax. She was born at a gestational age of 32.4 weeks with a birth weight of 3450 g. Postnatal pleural drainage showed a bilateral chylothorax. In spite of drainage and maximal ventilatory support, the patient died after 5 days from respiratory insufficiency. Post mortem examination showed prominent cystic pulmonary lymphangiectasis and pancreatic lymphangiectasis in the absence of cardiovascular anomalies. Identical to her half-sister, the second child also presented prenatally with a bilateral chylothorax. She was born at a gestational age of 31 weeks with a birth weight of 2600 g. She presented with severe respiratory insufficiency of which she died after 2 days. Post mortem examination showed and identical pathology, i.e. prominent pulmonary lymphangiectasis and pancreatic lymphangiectasis. Karyotypes of both patients and the mother were normal. A PTPN11 mutation (Noonan syndrome) was excluded in the first child.

CPL (MIM 265300) usually is a sporadic condition. Recurrence in siblings has been reported, suggestive of autosomal recessive inheritance. However, the present observation, with CPL in two female half-siblings is compatible with an autosomal dominant inheritance, with either gonadal mosaicism in the mother or, less likely, non-penetrance in the mother.

P0078. Molecular analysis of Connexin 26 (GJB2) gene among 114 Iranian families at risk of Hereditary Hearing Loss (HHL).

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Mutations in Connexin 26 (GJB2) gene are the common cause of Autosomal Recessive (AR) Hereditary Hearing Loss (HHL) in many populations. Molecular analysis of GJB2 gene performed, using ARMS/PCR and direct sequencing techniques, for 114 unrelated Iranian families which had affected individuals with HHL. Of these, GJB2 mutant alleles were found in 32 families (28% or 32/114 of the families) which most of them (i.e. 64% or 21/32 of the families with GJB2 mutation) were from North-western of the country. The most found common mutations were 35delG (75% or 48/64 of the found mutant alleles), W24X (6% or 4/64), R127H (5% or 3/64), IVSI+1 G>A (5% or 3/64), delE120 (4% or 2/64), R32H (1/64), 167delT (1/64), R184P (1/64) and 299delAT (1/64), respectively. Homozygosity for the 35delG mutation was the most common cause of HHL in the patients. Our finding of mutation types and their frequencies would be helpful for developing HHL mutation screening plan in Iran and other neighbor's countries.

P0079. Mutation analysis of the Connexin-26 gene in patients with ARNSHL in two provinces of Iran

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Mutations in GJB2 gene at the DFNB1 locus on chromosome 13q12 are associated with autosomal recessive non-syndromic hearing loss (ARNSHL) .There are many known mutations in this gene that cause hearing loss. A single frame shift, at position 35(35delG) accounts for 50% of mutations in Caucasian population with carrier frequency of 1.5-2.5%. In this study we investigated the prevalence of the GJB2 gene mutations using direct sequencing the coding exon of this gene within ARNSHL individuals from 53 families in two Iranian provinces. Eight different GJB2 variants were identified one of which (Deletion 16 nt at 176-190) was notpreviously reported for the Iranian population. Five GJB2 mutations including 35delG, 233delC, 176-191del16nt, W24X, L90P were found in 10 of 53 families (18.87%).Two different polymorphisms V153I and S86T were also found. One variant A171T with unknown effect also is detected. Six of 53 families were observed to have GJB2 mutations in both alleles (11.32%).The most common mutation was 35delG so that 3 (30%) out of 10 families that have GJB2 mutations contained 35delG mutation in both alleles and the frequency of 35delG allele is 0.50 between 10 out of 53 families.
Thus, contribution of GJB2 mutations appears less significant in familial deafness in Iran. This necessitates further assessment for the other known gene regions as well as search for new genetic factors in hereditary type of genetic deafness in Iranian population.

P0080. Associations of glutation-S-transferase M1 0/0 (GSTM1 0/0), GSTT1 0/0 and -1562 C/T matrix metalloproteinase 9 (MMP9 CT) genotypes with a risk and clinical features of COPD

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It is possible that a character of decline in lung function is a result of genetic susceptibility. COPD is defined by irreversible airflow obstruction. It pathogenesis is associated with xenobiotic metabolism and proteinases imbalance. The aim of study were I)to investigate frequencies of GSTM10/0, GSTT10/0 and MMP9CT in Russian COPD patients, II)to estimate their combined effects and III)their associations with quantitative spirometric indices (FEV1 and FVC in % of predicted value). It was examined 72 COPD patients and 39 heavy smokers without history of chronic bronchitis (Smokers). I)Frequency of GSTM10/0 was different between COPD (0.38) and Smokers (0.21) (OR 2.58; 95% CI, 1.16 to 5.75; P=0.04). Differences in distribution of MMP9CT and GSTT10/0 genotypes were not significant. II)We observed a combined effect of GSTM0/0 and MMP9CT conferred 8-fold higher risk of COPD. (14%vs.3%; OR 7.7, CI95% 1.1-53.3, P=0.05). III)Baseline spirometric examination has not showed differences of a lung function in carriers of genotypes described above in both groups. Over 5 years of follow-up examination, GSTM10/0 was associated with more decline of FEV1 in compare to GSTM1+ (28.9±4.0 vs. 38.4±3.3; p=0.052) and FVC (55.4±4.1 vs. 69.2±4.2; p=0.057). Retrospective analysis in COPD patients aged 35-45, 46-55 and 56-65 years showed more fast decline of lung function in carriers of GSTM10/0 vs. GSTM1+ in group aged 56-65 years: FEV1 31.87±5.16vs. 44.66±4.62 (p=0.050) and FVC 56.23±6.19 vs. 73.25±4.79 (p=0.056). These results suggest that GSTM1, but not GSTT1 and MMP9 genotypes are associated with the risk of disease and decline of lung function in COPD.

P0081. Results of routine diagnostics of Cornelia-de-Lange-Syndrome: Screening for point mutations and deletions in the NIPBL gene

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Cornelia-de-Lange-syndrome (CdLS) is a heterogeneous autosomal-dominant disorder characterized by typical facial features, growth retardation, developmental delay, upper-extremity malformations and a variety of other abnormalities. The prevalence of CdLS is estimated to be around 1/10,000. Most cases are sporadic, although several familial cases are described.
Since 2004 it is known that up to 50% of CdLS cases are caused by mutations in the NIPBL gene on chromosome 5p13. This gene consists of 47 exons and mutations were found in the entire coding region.
We introduced the mutation screening of NIPBL in 2005 and so far 23 patients were referred to our lab for testing of CdLS. To identify mutations, we established a DGGE-screening protocol for the rapid and economical detection of mutations in all 46 coding exons and the splice consensus sites of the NIPBL gene. We detected 5 causative mutations: one missense, two nonsense, one frameshift and one splice site mutation.
To find out if the low mutation detection rate is partly due to rearrangements resulting in deletion or duplication of one or more exons, we tested the NIPBL gene for copy number changes with the MLPA (multiplex ligation dependent probe amplification) technique. We detected no exon deletions in the 18 patients without point mutations.
Our data indicate that DGGE is a highly sensitive screening method for detection of mutations in the NIPBL gene. Exon deletions in NIPBL are not a common cause of CdLS.

P0082. Interaction among MTHFR, PAI-1 and ecNOS gene polymorphisms predict the severity of coronary artery disease in Turkish patients

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Genetic factors play a role in the onset of coronary artery disease (CAD). The objective of our study is to evaluate the single locus and combined effects of 3 different genetic polymorphisms (Methylene tetrahydrofolate reductase C677T polymorphism, plasminogen activator inhibitor 4G/5G polymorphism and endothelial nitric oxide synthase 3-27 base pair repeat polymorphism) on the presence and extent of CAD in patients with early onset coronary artery disease.
Materials & Methods: DNA samples were obtained from 102 consecutive patients with early onset CAD documented by coronary angiography. Severity of CAD in patients was stratified into 3 groups as 1, 2 or 3-vessel CAD. 102 control subjects were selected among subjects with angiographically normal coronary arteries. Information on standard risk factors was collected. Multifactor Dimensionality Reduction (MDR) analysis was performed to seek a model of coronary artery disease based on these 3 genetic polymorphisms.
Results: Single locus effects of the 3 polymorphisms were not significantly related to the presence or severity of coronary artery disease (p values for PAI-1 4G/5G, MTHFR C677Tand ecNOS 3-27 are 0.14, 0.26, 0.14 respectively). However when gene-gene interactions

were studied, the severity of disease was related to the frequency of high risk alleles (p= 0.01, Spearman R= 0.26), yet MDR and haplotype analysis did not detect a significant genetic model (p=0.24).
Conclusion: These 3 genetic polymorphisms are susceptibility loci and genotypes of these genes are neither necessary nor sufficient for the CAD to occur, but co-existence of high risk alleles may increase the severity of CAD.

P0083. A novel RyR2 mutation in a family with symptomatic and asymptomatic catecholaminergic polymorphic ventricular tachycardia.

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Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an autosomal dominant inherited disorder characterized by adrenergic induced polymorphic ventricular tachycardias and risk of sudden death. The human cardiac ryanodine receptor gene (RyR2) has been linked to CPVT and RyR2 mutations are considered to cause defective Ca2+ channel function.
A 65-year-old female was referred to our hospital because of recurrent syncope after physical and emotional stress. Routine cardiac examinations revealed no structural abnormality. A treadmill exercise test induced premature ventricular contractions and ventricular tachycardia. The administration of beta-blockers was effective in suppressing the arrhythmias. We performed genetic screening by DNA sequencing and a novel mutation c.6636A>C (p.2212Lys>Asn) in exon 43 of RyR2 was identified .The mutation predicts a amino acid substitution K2212N, located near the FKBP12.6 domain. Lysine on position 2212 is a highly conserved amino acid. This mutation was not detected in 100 healthy controls.
Subsequently we evaluated the patients' offspring. None of the family members demonstrated a history of exercise-induced complaints. The 43-year-old daughter carried the RyR2 mutation; however, no premature ventricular contraction or ventricular tachycardia was observed in exercise testing. The patient's granddaughter, a 13-year-old-girl, also carried the RyR2 mutation and exercise testing showed premature ventricular contractions and short runs of ventricular tachycardia. On beta-blocking drugs, repeated exercise testing showed no ventricular arrhythmia's. Exercise testing in familymembers in whom we did not find the mutation revealed no abnormalities. Our findings in this family with a novel RyR2 mutation confirm the importance of predictive testing of (asymptomatic) family members during childhood.

P0084. Cranioectodermal dysplasia. A case report

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Cranioectodermal dysplasia (CED) is an autosomal recessive disorder characterized by defects of ectoderm-derived structures and characteristic skeletal anomalies. We report on a 3-year-old girl with CED. She was born at 38th week of gestation due to IUGR and oligohydramnio and referred to the genetic clinic because of dysmorphic features. In patient´s history chronic renal failure associated with renal dysplasia and diabetes insipidus were noticed. On clinical examination developmental delay and low growth parameters (<3rd centile) were noticed.
The child had short upper and lower limbs, frontal bossing, ptosis of the eyelids, micrognathia, high palate , low set ears with abnormal ear helices, brachydactyly with skin syndactyly of the fingers, 2-3 toe-syndactyly, abnormal dermatoglyphic pattern,fine sparse hair and dysplastic nails. Radiologic examination revealed craniosynostosis of the right coronary suture, narrow thorax with dysplastic ribs, bowed femurs with abnormal upper epiphyses, short ulnae, brachydactyly , wide metacarpals, short and bowed tibiae, short metatarsals and triphalangeal hallux. The above findings were indicative of acromesomelic dysplasia with craniosynostosis.
On abdomen ultrasound kidneys were small and dysplastic. Brain CT scan showed additionally large cisterna magna and cortical atrophy. Karyotype was 46, XX and DNA analysis for FGFR genes was normal.
Patient´s mother terminated her next pregnancy at the 23rd week of gestation since the fetal ultrasound raised the suspicion of a similar clinical syndrome which was confirmed by the fetal histological report. CED is a very rare condition and accurate diagnosis contributes to the ahead of time genetic counseling.

P0085. Autosomal recessive nonspesific craniosynostosis which is not mapped to FGFR1-3, MSX2 and TWIST loci

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We report a consanguineous family manifesting multiple suture synostosis, exophthalmos, midfacial retrusion and relative mandibular prognatism, parrot-beaked nose and short upper lip without limb malformations. Complete pedigree structure consists of a total of 72 individuals who are descendants of a single ancestor couple. Clinical presentation of the affected cases is mostly resembled autosomal dominant Crouzon's syndrome (MIM # 123500) although intrafamilial variations are documented. However, the observation of six affected members who were born to two radiologically normal parents in three different branches of the same family considered autosomal recessive (AR) inheritance. Twenty five well examined individuals from three generations were used to test for a linkage to known disease loci. Homozygosity mapping excluded the following loci: FGFR1-3 on chromosomes 8p12, 10q26 and 4ptel, TWIST and MSX2 on chromosomes 7p21 and 5q35 respectively. Linkage analysis also excluded all of these loci assuming autosomal dominant inheritance with reduced penetrance. AR inheritance has been suggested as a likely mode of inheritance for this type of craniosynostosis in 1970's (MIM # 218500). However, this assumption was not further supported since accumulating number of pedigrees clearly showed autosomal dominant tranmission. Moreover, heterozygote disease causing mutations mainly in FGFR genes were also shown in many sporadic cases with unknown entity. Neither a locus nor a gene has been reported for AR form of this disorder as yet. Our study now provides an evidence to reconsider a recessively inherited mutation at an unmapped novel locus predisposing Crouzon-like craniosynostosis
(Supported by Hacettepe University Research Fund # 0201101010).

P0086. Craniofrontonasal Dysplasia

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We studied 2 affected sisters,aged 17 and16, with frontonasal dysplasia and craniosynostosis, born to Iranian nonconsanguineous parents.
The first child has marked hypertelorism, a broad bifid nose, low posterior hair line, with a widow's peak. She has neck webbing, rounded shoulders, abnormal clavicles. Her IQ is normal. The second child has the same problems plus widely spaced teeth.
Their parents are normal with no other pregnancies or offsprings. We found their paternal grand mother had the exact same features that our cases have. Surprisingly their father has none of the findings.
Parent to child transmission has been shown but there is a predominance of affected females. Autosomal dominant inheritance with some sex limitation seems likely.

P0087. A new craniosynostosis syndrome

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Craniosynostosis can be either classified as simple or multiple. When the sagittal suture is synostotic in conjunction with both coronal sutures, the membranous bone of the calvaria expands between the sutures, resulting in a characteristic lobulated skull configuration known as cloverleaf skull such as Carpenter Syndrome. We report on

a patient with bilateral multiple craniosynostosis involving the coronal and lambdoid sutures.The proposita was the second child born to a healthy non-consanguineous couple. The similar manifestations of the Carpenter Syndrome and our case were: Acrocephaly, Brachycephaly, Cloverleaf skull, Sloping forehead, Upturned nasal tip, Flat nasal bridge, Epicanthic folds, Hypertelorism, Shallow orbits, Bilateral ptosis,Partial syndactyly of the feet, Micrognathia, High-arched, Short stature, Obesity, AR. Except to the above characteristics, our patient had other manifestations included: Acral anomalies, Vertebral anomalies, High myopia, Proptosis, Anodontia. There were some typical features in Carpenter Syndrome which we could not find in our case including: Midfacial hypoplasia, Syndactyly, camptodactyly, Brachydactyly , clinodaclyly & Single flexion crease of the hands, Hypoplasia of the middle phalanges, Preaxial polydaclyly of the feet, Dysplastic ears, Low-set ears, Genital abnormalities, Sclerocornea, CHD, Mental deficiency, hearing loss. The features of our case were not typical of any known craniosynostosis syndrome. Search of POSSUM, LDDB and the medical literature uncovered no similar case. The constellation of manifestations in this patient suggests a previously unrecognized syndrome resembling Carpenter Syndrome. Therefore, we conclude that our patient may represent an extension of the Carpenter Syndrome or most probably a new Craniofacial Syndrome.

P0088. Low GABA concentrations in CSF in Crisponi syndrome: a sign of poor prognosis?

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In 1996, Giangiorgio Crisponi described a recessive syndrome characterized by recurrent hyperthermia, truncal hypotonia, facial contractions, camptodactyly and feeding difficulties. Phenotypic overlap with Schwartz-Jampel syndrome type 2 (Stüve-Wiedemann syndrome) exists. In the original report, Crisponi determined a low level of gamma-amino-butyric acid (GABA) in the cerebrospinal fluid (CSF) in an infant, who died at 12 weeks of age.

Our patients (two males and two females) are the offspring of three unrelated Turkish families. All children presented with perioral contractions, dyspnea on exertion and camptodactyly in the neonatal period. Bowing of the long bones was not observed. They developed truncal hypotonia and recurrent episodes of hyperthermia up to 42°C in the first month of life. Because of severe swallowing problems, all children needed a nasogastric feeding tube, which was followed by a percutaneous gastrostomy tube in two of the patients. In the most severely affected child, low levels of GABA were detected in the CSF at the age of 18 and 19 months, respectively. In the three other children, CSF-GABA levels were within the normal range. These three patients are surviving with mild developmental delay, scoliosis and dysautonomia symptoms. The child with the low GABA concentrations died at the age of 30 months.

We conclude that Crisponi syndrome can be distinguished from Stüve-Wiedemann syndrome by the absence of bowing of the long bones. Our data indicate, that low levels of GABA in the CSF of children with Crisponi syndrome might be associated with a poor prognosis.

P0089. Crouzon syndrome: Twelve new Greek patients clinical and molecular investigations

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Crouzon syndrome (CS) is a well-recognized disorder characterised by cranial synostosis, shallow orbits with proptosis and maxillary hypoplasia. It is inherited with an autosomal dominant pattern with high penetrance and variable expressivity, while 44-67% of cases represent new mutations. We present clinical and molecular data of 12 Greek patients aged 2 months-36 years with a clinical diagnosis of CS. Nine cases were sporadic and three familial. The most consistent findings were craniofacial dysmorphic features (100%), followed by neurological (34%) and gastrointestinal anomalies (25%). Exposure conjunctivitis, poor visual acuity and optic atrophy were less frequent complications (18%) than shown in the literature. Midface hypoplasia contributed to the development of high-arched palate and probably explains the high frequency (55%) of mouth breathing and tooth crowding. Congenital heart defects were noticed in 9%, while only one patient had acanthosis nigricans involving eyelids and the skin of the neck. Five patients (42%) presented with mental retardation. Molecular analysis by DGGE and direct sequencing in exons 8,10 of FGFR2 and exons 7,10 of FGFR3, revealed mutations in 8 cases. Among the 3 FGFR2 mutations that we identified, two were missense substitutions (Cys342Tyr, Gly338Arg) and one patient was compound heterozygote of Phe276Cys/Val277Ala. The most frequent missense mutation in the transmembrane domain of FGFR3 was Ala391Glu, while one patient carried the P250R in exon 7 of FGFR3. In all familial cases, the origin of mutations was maternal. Detection of the molecular defect in CS allows phenotypic-genotypic correlation, proper genetic counseling and prenatal diagnosis.

P0090. Subtelomeric FISH analysis in 80 patients with mental retardation

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Mental retardation (MR) affects approximately 1 to 3% of the general population. The etiology is still poorly understood, and it is estimated that one-half of the cases are due to genetic factors. Cryptic subtelomeric aberrations has been found in roughtly 5 to 7% of all cases. We performed a subtelomeric FISH analysis in 80 unrelated mentally retarded children with normal standard karyotype ascertained by a checklist that evaluates the grade of developmental delay, dysmorphisms, growth defect, and associated congenital malformations. Nine cryptic chromosomal anomalies have been identified (11,25%): four de novo deletions of 1p, 6q, 7q, 11p, respectively, three unbalanced translocations of parental origin, der(20)t(16q24;20q13.3)pat; der(6)t(6;1) (p22.3,q44)mat, and a der(7)t(7;12)(q34;q24.32)mat, two de novo unbalanced translocations: t(6pter;6qter) with partial 6q trisomy and 6p monosomy and t(5;10)(pter;qter) with 10q partial trisomy and 5p partial monosomy. Clinical features of most of these patients are consistent with the corresponding new emerging chromosome phenotypes, further supporting the role of cryptic subtelomeric analysis in the work-up of children affected by mental retardation.

P0091. Estimation of cystic fibrosis mutation dF508 frequency in Latvia.

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Previous investigation of the mutation frequency in pure Latvian individuals (Krumina et al., 1996), has shown that the average frequency of dF508 mutation is 1:42. The estimation of the expected CF incidence in the Latvian population showed that the frequency of non-mild CF cases in newborns should be 1:3300. The average number of newborns per year in the period 2000 to 2004 was 20260. So, the expected number of CF patients should be 6 per year. However in this 4-year period only 5 new CF patients were registered. This discrepancy we wanted to explain by the ethnic diversity of Latvian inhabitants (Russians-28.6%, Belorussians-3.82%, Ukranians-2.55%, Lithuanians-1.37%). In these populations, the frequency of dF508 mutation is less common.

The aim of this study was to detect the frequency for dF508 mutation in the population of Latvia. 124 individuals were investigated (Latvians-59.67%, Russians-30.64%, Belorussians-3.22%, Ukranians-3.22%, Poles-3.22%). DNA diagnosis was performed using phenol-ethanol DNA extraction method and PCR technique. Four individuals were found to be carriers of the dF508 mutation. Consequently, the heterozygote frequency for the population of Latvia for CF dF508 mutation is 1:31.

In the beginning of the study it was expected that dF508 mutation frequency in the population of Latvia is less than previously reported. Instead, the mutation was found at a higher rate than it was expected.

Since the expected number of CF patients in Latvia is much greater that are presently being diagnosed, the likely explanation is that CF in Latvia is being misdiagnosed.

P0092. Genotype-phenotype correlation in cystic fibrosis associated liver disease- is it real?

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Cystic fibrosis(CF) is the most frequent monogenic disease in Caucasian population, potentialy lethal, with marked clinical variability. Hepatobiliary manifestation consists in: elevation of liver enzymes, cholestasis, hepatosteatosis, focal biliary cirrhosis, multilobular cirrhosis, cholelithiasis, microgallbladder. Liver disease is frequently associated with male gender, history of meconium ileus, increase age and severe genotype; therefore questions concerning a genotype-phenotype correlation.

Aim study: evaluate the genotype-phenotype correlation in patients with cystic fibrosis associated liver disease. 85 patients were followed up by clinical assessment, genetic tests(ElucigeneTM CF29 kit), biochemical markers, ultrasound examinations. 42% had hepatobiliary disease; in 56 children younger then 10 years, only 11% had elevation of liver enzymes, 2 associated neonatal cholestasis and jaundice (ΔF508 homozygous).28% of children (over 10 years) had liver disease; in about 8 % of the patients, multilobular cirrhosis was diagnosed, in 4 confirmed at biopsy. Genotype structure of 85 patients revealed 32 ΔF508 homozygous genotypes, 22 - ΔF508/X, 5 - non - ΔF508/X and 26 unknown (X/X), the frequency of ΔF508 allela being 50, 58%.Severe liver disease developed in 7 patients, 4 children-ΔF508 homozygous genotype, 1 with 2183AA >G/621+1G>T, 1-ΔF508/G542X, 1-G542X/x. Between 32 ΔF508 homozygous genotypes, only 13 had a hepatobiliary disease.

We could not establish a correlation of phenotype and a specific mutation. Different evolution of cases with same genotype remains unexplained, suggesting that "genetic modifiers" and/or environmental factors influenced disease expression. (Study performed through Grant Research Programme-CNCSIS A/1188/2004)

P0093. Detection of mutations in patients suspected of Cystic fibrosis

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Cystic fibrosis (CF) is the most common severe inherited disease in Caucasians, occurring in approximately 1 in 2500 births. It results from mutations in the CFTR gene, a transmembrane chloride ion channel. The defect in the chloride channel leads to a viscous mucous production which, in turn leads to pathology in primarily three organ systems. The classic form of CF involves chronic pulmonary disease, pancreatic insufficiency and infertility. Although there is wide range of clinical expression, most individuals with CF experience substantial morbidity and require lifelong care.

There is no data of this mutation and frequencies of carriers in Armenian population. In 55 patients with a clinical suspicion of CF molecular testing has been performed for 25 frequent mutations and 8 polymorphisms. The genotyping analysis of these patients revealed mutations in 8 cases. The detected mutation are ΔF508(1 case), and 3659delC ; 2184delA in compound heterozygote (7 cases). There have also been detected Poly(T)-5,7 or 9 polymorphisms. The important difference with Poly(T) testing is that the role of 5T depends on its relationship with R117H and that 5T variant alone was not usually associated with classic CF. It is important testing in CF carrier screening, because if one parent has R117H and 5T together on one chromosome and the other parent carries another CF mutation, their children are at risk for having classic CF.

P0094. Phenotypic and genotypic peculiarities of cystic fibrosis in Republic of Moldova

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Background. CF is one of most frequent monogenic diseases in Caucasians.

Methods. Was analyzed correlation of genotype and phenotype of 70 patients with cystic fibrosis (CF) from 2 months to 24 years, 43 male, 27 female.

Results. By polymerase chain reaction and restriction fragment length analysis were identified the following mutations: DF508 - 50%, including in 14.3% of cases homozygote and in 35.4% -heterozygote, and R334W - 4.3%.

DF508 is associated with pancreatic insufficiency (100% homo, 88% in heterozygous). In all homozygous and in 70% heterozygous was revealed severe course of disease. In 30% of patients with DF508 were revealed cases of chronic colonization of sputum by Ps. aeruginosa. In 35% was decreased nutritive status. The mutation of 7 exon, R334W, was founded in 4.3% of cases in compound heterozygote status, with relative mild course of disease. Was revealed homozygote by metH polymorphism, and, despite the early debut in 3 months-old patient the disease slow progressing, at present the age of patient is 17, and status is stable.

In 44.3% the mutations were not identified, in 28.6% of these patients suffered from mixed form of CF, 11.4% from pulmonary form and 4.3% from intestinal form of CF. The course of disease were severe in 12.9% of patients and moderate in 31.4%.

Conclusion. DF508 which present in 50% of patients determine severity of the disease and life span of patients with CF from Moldova. The high part of non-identified mutations suggest necessity to improve molecular diagnosis of CF in Moldova.

P0095. Phenotypic variability in Dandy-Walker complex

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Background: The Dandy-Walker malformation is a rare congenital anomaly characterized by vermis agenesis or hypoplasia, cystical dilatation of the 4th ventricle and a large posterior fossa. The syndrome is defined by the mere presence of these three signs. The Dandy-Walker complex (DWC) includes cystic malformations of the posterior fossa, such as Dandy-Walker malformation, Dandy-Walker variant, mega cysterna magna and posterior fossa arachnoid cyst.

Objective. Presentation and discussion of three cases with different morphologic and clinical forms of the Dandy-Walker complex. 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 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 gastroesophageal reflux. Patient #3 was diagnosed with Dandy-Walker variant in a rare association with neurofibromatosis.

Conclusions. Dandy-Walker complex is a malformative association of the central nervous system. Its clinical, radiological and functional manifestations are variable and require adequate diagnostic and therapeutic measures.

P0096. Low sperm motility due to mitochondrial DNA multiple deletions

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There is increasing evidence that mitochondrial DNA (mtDNA) anomalies in sperm may lead to infertility. Point mutations, deletions and depletion have been associated with decline of fertility and motility of human sperm. It has been proposed that mtDNA genetic alterations can also be responsible for sperm dysfunction. Sperm motility is one of the major determinants of male fertility and is required for successful fertilization.

It is becoming increasingly evident that both point mutations and large-scale deletions may have an impact on sperm motility and morphology. In this study we investigated association between occurrence of mtDNA Δ4977 bp deletion with diminished fertility and motility of

human spermatozoa. The possible relationship between multiple deletions of mtDNA and the decline of fertility and motility in human spermatozoa was further explored in 50 subjects including sub-fertile and infertile males. Our results showed that the ratio of the deleted mtDNA in the spermatozoa with poor motility and diminished fertility were significantly higher than those in the spermatozoa with good motility and fertility. Our findings suggest that mutation and deletion may play an important role in some pathophysiological conditions of human spermatozoa.

P0097. Deletion of the short arm of chromosome 18-case presentation

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The objective of the presentation is a case of holoprosencephaly in a newborn with structural anomaly of chromosome 18.The diagnosis was established on the basis of clinical history,clinical examination,caryotyping and imagistic investigations.There is a case of female newborn of 36 weeks of gestation.In clinical examination there is a craniofacial dismorphism,with microcephaly,low implantation of the ear lobe,celocephaly and proboscis nasi.A respiratory failure is present due to surfactant deficiency and nasal anomaly.Cerebral sonography confirms the presence of a single ventricular chamber of increased dimensions,separated cervical plexus and separated thalamic nuclei. Radiography of the facial bones confirms the absence of nasal septum and the caryotype analysis (G-banding) confirms female gender and deletion of the short arm of chromosome 18(46,xx,18p-).Based on the above presented data,the case was diagnosed as non-lobar holoprosencephaly with the deletion of the short arm of chromosome 18 and respiratory failure.The only therapeutic care was the palliative one,in a severe cerebral anomaly with associated mental retardation.
Key word:newborn,holoprosencephaly,chromosome 18 deletion

P0098. Female with partial Turner syndrome, normal menstruation, deletion Xp22.33, and duplication Xp22.12-22.32 analysed using array-based Multiplex Amplifiable Probe Hybridisation (array-MAPH)

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Mosaics and partial deletions or duplications of the chromosome X result in different degrees of the Turner syndrome. Minimal deletions of chromosome Xp including *SHOX* (short stature homeobox-containing gene) can cause short stature, short metacarpals, cubitus valgus, Madelung deformity, high arched palate, and/or short neck. The proposita was born at term, weight was 2,300 g and length 45 cm. At the age of fourteen years she consulted a geneticist because of short stature. Height was 140.2 cm (-5 SD), weight 43.5 kg (-2 SD), and OFC 54 cm (-0.5 SD). Findings included low occipital hairline, numerous pigmented nevi, Madelung deformity, normal pubertal development and growth hormone deficiency. Karyotyping from peripheral blood revealed a chromosome Xp rearrangement. Fluorescent in situ hybridization (FISH) showed a complex aberration with a distal Xp22.3 deletion including the *SHOX* gene and a proximal Xp22.3 duplication including the *STS* gene. Array-based multiplex amplifiable probe hybridization (array-MAPH, Patsalis et al. EJMG 2005; 48:241-9) revealed a duplication spanning from Xp22.12 to p22.32. Array-MAPH results were confirmed using FISH with BAC clones. The final karyotype was 46,Xadd(X)(p) ish der(X)(wcpX+,SHOX-,STS++,RP11-431J24++,RP11-160F21++,RP11-326G24++,RP1-60N8++,RP11-88F09++,RP11-406A18++,RP11-261M11+,DXZ1+). In conclusion, the proposita has a rare complex rearrangement of the distal part of chromosome Xp. The case demonstrates that array-MAPH is a powerful and convenient technique for analyzing small complex chromosome rearrangements.

P0099. Denys-Drash syndrome and gonadoblastoma in a patient with Klinefelter syndrome

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Denys-Drash syndrome (DDS) is characterised by the triad of congenital or early-onset nephropathy, male pseudohermaphrodism and Wilms' tumour. It is caused by constitutional heterozygous exonic point mutations in *WT1*. We report a phenotypically female child with congenital nephropathy associated with end-stage renal failure, Klinefelter syndrome (47, XXY) and a heterozygous missense mutation (c.1180C>T, p.R394W) in the *WT1* gene. She underwent nephrectomy because of the risk of Wilms' tumour, and gonadectomy because of the risk of gonadoblastoma. Histology of her kidneys revealed mesangial glomerulosclerosis and the presence of dysplastic tubules with no evidence of Wilms' tumour. Examination of the dysgenetic gonads revealed ovarian stroma showing bilateral unifocal gonadoblastomas. To the best of our knowledge this is the first reported patient of DDS with Klinefelter syndrome. Gonadoblastoma has been previously reported in association with DDS.

P0100. Prevalence of LIM Domain-Binding 3 (LDB3) gene mutations in idiopathic dilated cardiomyopathy.

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Familial Idiopathic dilated cardiomyopathy (IDCM) is a genetically heterogeneous. Disease. Among disease-causing genes, LDB3 (MIM+605906) mapping at 10q22.2-q23.3) has been recently reported as causally linked to both non-compaction left ventricle (NCLV) and IDCM. DB3 encodes a protein that is a component of the Z-line in both skeletal and cardiac muscle. Recent studies have demonstrated that LDB3 knock-out mice develop cardiomyopathy and that defects of the gene may cause familial IDCM. Six mayor cDNA isoforms of LDB3 have been identified in human striated muscle and are generated by alternative splicing of a single gene.
We screened the LDB3 gene in 115 unrelated index patients diagnosed with IDCM according to WHO criteria. The gene screening was performed by denaturing high performance liquid chromatography (DHPLC) and bidirectional sequencing of hteroduplex amplicons.
Five mutations were identified in six probands (5,21%): D117N (2 unrelated probands), S196L (one proband), T358A (one proband), T357I (one proband), V588I (one proband) (known mutations are in bold). The Val588Ile, although reported as rare polymorphism, was absent in 120 healthy controls. None of the patients showed echocardiographic features suggestive for NCLV according to Chin et al. and McKenna et al. criteria.
We confirm LDB3 gene as candidate gene for familial IDCM, independently on echocardiographic pattern of NCLV. The prevalence of the LDB3 gene mutations in a consecutive series of more than 100 patients is 5%. Despite the description of NCLV as specific feature associated with LDB3 gene mutation the clinical phenotype did not show specific clinical markers.

P0101. Mutational analysis in a family with Autosomal Dominant Optic Atrophy (ADOA).

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Autosomal dominant optic atrophy (ADOA) is the most prevalent hereditary optic neuropathy
resulting in progressive loss of visual acuity, centrocoecal scotoma and bilateral temporal atrophy of the optic nerve with an onset within the first two decades of life. Genetic linkage analyses localized a dominant atrophy gene (OPA1) to chromosome 3q28-qter, and mutations of the OPA1 gene in familial ADOA were identified.

We present an ADOA Italian family with four related affected females. The purpose of this study was 2-fold: to determine the types and frequency of matations in OPA1 which caused ADOA in our family, and to determine whether a second condition, Leber's hereditary optic neuropathy with a similar pathology to ADOA, could also be caused by mutations in OPA1.
For this, initially we screened the proband and the family members for the 3460A, 11778A, and 14484C LHON mutations by PCR amplification followed by mutation-specific restriction endonuclease digestion, but this search was negative. The analysis of the entire coding region of the OPA1 gene by direct sequencing of PCR- amplified exons in all familial cases revealed four polymorphisms described previuosly : 473G in exon 4, 2109T in exon 21, +51G, and +25A. The molecular analysis of other asymptomatic members of family confirmed the same polymorphisms.
These data suggest that OPA1 gene is not involved in ADOA in our family.
Further studies are required to identify the causative ADOA gene in this family and to delineate the role of this locus.

P0102. The meiotic stage of chromosome 21 nondisjunction as indicated by STR polymorphic markers among Down Syndromes in Iran

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Down syndrome is one of the main causes of mental and growth retardation mainly happened due to chromosome 21 nondisjunction. This is the first study in Iran categorizing cases of Down syndromes by parental origin and stage of meiotic error of chromosome 21. We studied 224 families having a child with Down syndrome using conventional cytogenetic analysis and chromosome 21 specific STR markers (D21S11, D21S1414, D21S1440, D21S1411, D21S1412). Parental origin and stage of meiotic error were studied using five STR markers. Meiotic nondisjunction of chromosome 21 was studied in 202 cases with free chromosome 21. The parental origin and the meiotic stage of chromosome 21 nondisjunction were detected in 190 of cases. Parental origin of nondisjunctions were derived as 167 (88%) maternal and 23 (12%) paternal. In maternally derived cases, meiotic I nondisjunction was observed in 121 (64%) and meiosis II in 46 (24%) of cases whereas in paternally derived cases meiosis I was detected in 13 (7%) and meiosis II in 10 (5%) cases. There was no significant difference in the distribution of maternal ages between maternal meiosis I error versus maternal meiosis II error. It is unexpected that a nondisjunction at especial of maternal ages between maternal meiosis I error against maternal II error, related significantly to the rising incidence of Down syndrome with advancing maternal age. This data is usable in analysis of maternal and paternal genetic or environmental risk factors to understand better the cause of chromosome 21 trisomy.

P0103. The partial trisomy for the distal short arm of chromosome 6 (region pter→p21) in a girl with psychomotor development delay and dysmorphic features

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We report a girl presenting a de novo partial trisomy for the distal short arm of the chromosome 6. Our patient is a two-year-old girl, first child of healthy non consanguineous parents from complicated pregnancy with symptoms of miscarriage. The dysmorphic features were seen from the birth of this girl. Facial dysmorphism is characterized by a microcephaly, craniosynostosis, facial asymmetry, high, prominent forehead with depressed frontal bone on the right size, ocular hypertelorism, blepharophimosis/short palpebral fissures, ptosis, flat nasal root, very short nose, long philtrum, thin lips, small mouth, high arched palate, simple, low-set pinnae with poorly developed lobes and small chin. Unusual dermatoglyphic changes are identified on the girl's palms. The nipples are hypoplastic and abnormally placed from each other. There are expressed hirsutism on the pubic bone area. The short stature attends this partial trisomy.
Clinical follow-up showed these clinical findings: CT scan showed mild hydrocephaly, X-ray - dysplastic ribs with partially accretion. Cardiac ultrasonography showed stenosis of a. pulmonalis, dilatation right size of the heart. Renal abnormalities have included hypoplastic kidney with renal dysfunction.
In our patient we detected a de novo duplication of the short arm of chromosome 6 identified on a 400 band chromosomal analysis. Cytogenetic analysis was performed from GTG banded metaphases. The girl's karyotype was 46, XX, dup(6)(pter→p21). Parental karyotypes were normal.

P0104. Dystrophic epidermolysis bullosa pruriginosa in Italy: molecular characterization and pathogenetic aspects

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Dystrophic epidermolysis bullosa pruriginosa (DEB-Pr) is a rare variant of DEB due to COL7A1 dominant and recessive mutations which is characterized by severe itching and lichenoid or nodular prurigo-like lesions mainly involving the extremities. Less than 20 patients have been described showing variable disease expression and, frequently, delayed age of onset. We report the clinical and molecular characterization of 7 Italian DEB-Pr patients, 3 with recessive DEB-Pr (RDEB-Pr) and 4 with dominant DEB-Pr (DDEB-Pr). In all patients the signs were typical of a mild DEB phenotype, until the pruritus onset, after which the distinctive skin lesions of DEB-Pr appeared. Nine mutations were disclosed in COL7A1, 5 recessive and 4 dominant. These mutations allowed in all patients the production of a given amount of partially functional type VII collagen (COLLVII), detected at the dermal-epidermal junction (DEJ). Three mutations were novel, and one arose *de novo*. Furthermore, 2 unrelated RDEB-Pr patients were carrying the recurrent c.7344G>A Italian mutation and 2 unrelated DDEB-Pr patients were carrying 2 different mutations in intron 87, leading to the in frame skipping of exon 87. In order to find factors involved in the pathogenesis of DEB-Pr, we analysed the patients skin for the presence of Igs by direct immunofluorescence. In a patient IgG and C3 linear deposits along the DEJ were present, in another IgM deposits were detected in the same location. These results underline for the first time the possible involvement of immunological factors, likely an antibody-mediated autoimmune reaction, at least in some DEB-Pr patients.

P0105. The genetic causes of early onset hereditary hearing loss among Estonian children

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During last 6 years (2000-2005) 119 children with early onset hearing loss, as a main complaint, have been referred to genetic counseling. Eighty five percent of them had moderate to profound and 9% mild bilateral hearing loss (sensorineural, conductive or mixed); 6% of patients had unspecified hearing loss.
Careful clinical investigation was performed in all of them for excluding syndromic hereditary impaired hearing (HIH). Since 1999 we have investigated 35delG mutation in *GJB2* gene, which encodes the gap junction protein connexin-26. Since 2005 we have investigated the genotype of the children with HIH by arrayed primer extension (APEX) method, which covers 201 mutations in 8 genes (6 nuclear genes: *GJB2,* Connexin-30, Connexin-31, Connexin-43, Prestin and Pendrin gene, and 2 mitochondrial genes: 12S-ribonuclear-RNA and the transfer RNA for serine gene).
Thirty eight patients (32%) were homozygous for 35delG mutation in *GJB2* gene, 15 (13%) were heterozygous for 35delG mutation, and in 9 of them the mutation in the second allele has already been identified (35delG/R143W, 35delG/167delT, 35delG/M34T, 35delG/

312del14, and 35delG/IVS1+G>A genotypes). One patient was compound heterozygote for R143W/M34T mutations in *GJB2* gene. In one patient with profound bilateral sensorineural hearing loss M34T mutation was found in one allele of *GJB2* gene and F335L mutation in one allele of Pendrin gene. In 6 patients syndromic HIH was identified (branchio-oto-renal, Leopard, Stickler, Klippel-Feil syndrome and neurofibromatosis).
In summary, 55 patients (46%) the exact etiology of HIH was identified, in 49 (42%) of them nonsyndromic HIH and in 6 (5%) of them syndromic HIH.

P0106. Mutations in the EDAR gene are not uncommon among patients with hypohydrotic ectodermal dysplasia.

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Ectodermal dysplasia is a genetic heterogenous disorder characterized by malformation of teeth, sparse hair and the lack or reduced number of sweat glands. Mutations in the ED1 gene cause X-linked ectodermal dysplasia.
Autosomal forms result from mutations in either the EDAR gene or the EDARADD gene. There have been only a few publications reporting mutations in the EDAR gene. To evaluate the contribution of EDAR mutations, we analysed 8 families with clinical symptoms of ectodermal dysplasia so far. All patients where previously tested negative for ED1 mutations.
In a large german kindred with 9 affected family members the mutation Arg420Gln was detected in EDAR. The index patient is the youngest affected member of the family. She has only six molars, which was the reason to finally ask for genetic counselling. Missing front teeth and reduced ability to sweat are known in this family. Only the affected grandmother shows very thin hair. In this family the phenotype of ectodermal dysplasia is variable.
In a sporadic case we detected a novel compound heterozygous point mutation: Glu379Lys inherited from the father and the splice site mutation IVS5+1ds G>A inherited from the mother. Neither the mother nor the father show clinical features of ectodermal dysplasia suggesting a recessive trait.
Finally, in another german family the novel dominant mutation 1165delGA was detected.
In conclusion we think that mutations in the EDAR gene are more common than it was thought. Testing for EDAR mutations should be considered in all cases tested negative for the X-linked form.

P0107. Familial ectopia lentis in three generation

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We report a case of ectopia lentis in a three-generation family in which the proband has a less severe ocular phenotype and a few manifestations of Marfan syndrome in the skeletal system. **Objectives**: to describe and compare clinical manifestations of ectopia lentis in younger and older generations; to study the phenotype-genotype correlation. **Patients and Methods**: five affected persons from three generations were investigated; evaluation included physical examination and a detailed family history. Ocular examination included visual acuity and visual field testing, retinoscopy and refraction, slit lamp examination, fluorescein angiography. **Results**: the clinical expression of lens dislocation was variable in this family. The family pedigree showed an autosomal dominant mode of inheritance with complete penetrance and variable expressivity. Consanguinity was not present in this family. Family history was remarkable because grandmother and mother's siblings of our patient expressed severe phenotype of marfan syndrome-associated ectopia lentis (severe ocular, musculoskeletal, cardiovascular and dermatological manifestations). The patient and his mother shared similar phenotype (less severe than their relatives). All cases were defined by clinical signs and submitted to surgery for correction of ectopia lentis. **Conclusions**: ectopia lentis is a hereditary condition with clinical variation; surgical results depend on the severity of the phenotype; molecular-genetic data should be integrated with the corresponding clinical findings.

P0108. EEC syndromes and p63 mutation

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Ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome is a rare autosomal dominat multiple congenital anomaly syndrome with variable expression and penetrance. The most common clinical features are ectodermal dysplasia, ectrodactyly (distal limb anomaly), cleft lip/ palate, lacrimal duct and urogenital anomalies with usually normal mental development. EECs has been shown clinical and genetic heterogeneity and linked to chromosome 7q11.2-q.21.3 (EEC1), chromosome 19 (EEC2) and to chromosome 3p27 (EEC3) where heterozygous p63mutations were detected in unrelated EEC families. The p63 gene is a transcriptional co-activator and is of crucial importance for correct development of the limbs, ectodermal appendages (skin, nails, teeth, hair, glands), lip and palate. Several autosomal dominantly inherited human syndromes have recently been shown to result from mutations in the p63 gene. These syndromes have various combinations of limb malformations fitting the split hand-split foot spectrum, orofacial clefting, and ectodermal dysplasia with some overlapping features. The p63 syndrome family includes the EEC syndrome, AEC syndrome (eyelid adhesion/ ankyloblepharon-ectodermal dysplasia-clefting), ADULT syndrome (acro-dermato-ungual-lacrimal-tooth), Limb-mammary syndrome (LMS), and non-syndromic split hand/split-foot malformation (SHFM). The specific pattern of heterozygous mutations is distinct for each of these syndromes and the functional effects on the p63 proteins also vary as well. In all of these syndromes, the mutation appears to have dominant negative effect with gain of new function. Four isolated cases quoted from the joint- cleft clinic, with a wide clinical spectrum of EEC Syndrome and their p63 gene screening, including one mutation result, will be presented.

P0109. Intrafamiliar phenotype heterogeneity associated with p63 mutation - case report

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Ectrodactyly-Ectodermal dysplasia-Clefting syndrome (EEC) is a rare AD condition with variable expression, results from specific mutations in the p63 gene located on 3q27. Ectrodactyly is the defect in midportion of hands and feet. Ectodermal dysplasia consists of anomalies of skin and its adnexa, partial anodontia, microdontia, blue irides, photophobia, defects of lacrimal duct system. Clefting include cleft lip and/or palate. Another anomalies include congenital defects of genitourinary tract.
We report the case of a 35 years old pregnant woman with cleft lip and palate, hypacusis, photophobia, defect of lacrimal duct system, sparse hair, ectrodactyly of hands and feet, nail dysplasia. Prenatal ultrasound diagnosed in the fetus cleft lip, left hand ectrodactyly and mild unilater hydronephrosis. Mother decided to continue the pregnancy, which was uneventfull. The boy was born at term, without any afterbirth complications. He had cleft lip and palate, sparse hair, nail dysplasia, deficit of the 2nd finger and syndactyly of the 3rd and 4th fingers on his left hand. There were no other abnormalities on the extremities. Subsequent mutation analysis of the p63 gene of mother and her newborn son, performed after delivery, revealed heterozygosity for a mutation in exon 5 (R204Q) This mutation has previously been described in association with EEC syndrome.
Our case documents intrafamiliar phenotype heterogeneity associated with p63 mutations.
The parents are planning further pregnancy and they decided to terminate it in case of handicapped fetus. Discovery of the causal mutation enables us to perform prenatal diagnosis of EEC syndrome.
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P0110. New cases with ectrodactyly, ecdodermal dysplasia and macular degeneration syndrome

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EEM Syndrome (ectodermal dysplasia, ectrodactyly, macular degeneration) is a very rare condition characterised by ectodermal dysplasia, ectrodactyly and macular dystrophy. In addition to this a number of other abnormalities such as alopesia, cataract, absent eyebrows, oligodontia are the syndrome. The entity was defined as the EEM syndrome. The ocular fundus is identical for EEM syndrome which is separated from the other ectrodactyly syndromes. We report one brothers and one sister with EEM syndrome born to consanguineous parents. In contrast to other ectrodactyly syndromes autosomal recessive inheritance is most likely. The proband is a 34 year old woman. Her parents were first cousins and had no signs of ectrodactyly or ectodermal dysplasia. Her brother of the proband was 36 years old with clinical features of EEM similar to those of the first proband upon examination. One of their brothers having the features of the EEM syndrome also died at 45 years of age. The following reports of a brother and sister contribute to the existing knowledge on this rare syndrome, and may help further identify its features.

P0111. A cellular test for Ehlers-Danlos syndromes diagnosis

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Ehlers-Danlos syndromes (EDSs) are hereditary connective tissue disorders with clinical, genetic and allelic heterogeneity. In the six EDS forms, mutations in genes encoding type I, III and V collagens (COLI, III, V), lysyl hydroxylase, ADAMTS-2 and tenascin X have been reported. EDS patients clinical diagnosis, following the Villefranche Nosology criteria, must be confirmed at molecular level. Protein and mutation characterization are performed starting from cultured patients skin fibroblasts. We report a cellular test for EDSs fibroblasts based on the immunofluorescence analysis of a panel of proteins involved in the formation and organization of the extracellular matrix (ECM) of cultured cells. Skin fibroblasts derived from 27 patients affected with the different EDSs showed the absence of fibronectin (FN) and fibrillin1 (FBN1) ECM, disorganization of their receptor, the α5β1 integrin, and organization of the alternative αvβ3 integrin receptor ($FN^-FBN1^-α5β1^-αvβ3^+$). Six control fibroblasts strains showed an $FN^+FBN1^+α5β1^+αvβ3^-$ phenotype. Fibroblasts derived from other connective tissue disorders (15 strains), showed different patterns and combination these proteins organization. Therefore, the $FN^-FBN1^-α5β1^-αvβ3^+$ phenotype seems to be specific for EDS fibroblasts. In addition to these markers, specific alterations were identified in the different EDSs, *i.e.* lack of: COLV- and COLIII-ECM in classic EDS; COLIII-ECM in vascular EDS; tenascins in hypermobile EDS; COLI and COLIII in kyphoscoliotic EDS; COLI-, COLIII- and COLV-ECM in arthrochalasic EDS. Further analysis on a larger number of EDS cells mutants will allow to verify if these markers are a common feature and may address the molecular analysis.

P0112. Using Short tandem repeats for carrier detection and prenatal diagnosis of factor VIII gene in Iranian population

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Background: Patients affected with hemophilia A are due to lack of factor VIII protein in the coagulation cascade. Factor VIII gene is located on Xq28 and comprises 26 exons. There is a need for genetic counseling despite the improvement of replacement therapies. Using polymorphic markers in linkage studies would help in carrier detection and prenatal diagnosis. In this report we investigated the frequency of two short tandem repeats on factor VIII gene in Iranian population.
Methods: DNA amplification of 70 unrelated individuals (93 X chromosomes) was performed. Two repetitive regions on factor VIII including one in intron 13 and the other in intron 22 were amplified and resolved on 10% polyacrylamide gel and detected by silver staining.
Results: Different allelic bands were used as markers. These markers were made by the recovery of each band and T/A cloning of them. Studies have shown that there are six (18-23) allelic markers for intron 13. Sequencing analysis of intron 22 had shown two repetitive units including AG (6-7) and GT (16-18). This made it polymorphic but the differences between each unit were identified by direct sequencing or SSCP.
Conclusion: Haplotype analysis of these intragenic dinucleotide repeats has shown to be more informative than RFLP analysis and could help us to provide carrier detection and prenatal diagnosis for familial cases. Although dinucleotides are polymorphic they may lead us to genotyping errors of heterozygotes and homozygotes in the studied population.

P0113. The neonatal exons 24-32 *FBN1* region: Clinical and mutation type analysis from an international series of 1057 probands with a pathogenic *FBN1* mutation

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Mutations in the *FBN1* gene cause Marfan syndrome (MFS) and a wide range of overlapping phenotypes. The severe end of the spectrum is represented by neonatal MFS (nMFS), the vast majority of probands carrying a mutation within exons 24-32. To assess patients carrying a mutation in this so-called neonatal region, we compared the clinical and molecular characteristics of 207 probands (20%) with a mutation in exons 24-32 with patients carrying a mutation in other exons from a series of 1057 probands. 25% of patients with a mutation in exons 24-32 presented a nMFS phenotype. Patients with a mutation in exons 24-32 presented earlier manifestations than patients with mutations located elsewhere ($p<0.0001$), including younger age at diagnosis of MFS (50% at 8 years vs 25 years), at diagnosis of scoliosis (50% at 16 years vs 30 years), ectopia lentis (50% at 22 years vs 33 years), aortic dilatation (50% at 13 years vs 30 years), aortic surgery (50% at 37 years vs 46 years) and shorter survival (79.4% at 30 years vs 98.8%). Similar results were found if patients with neonatal MFS were excluded. Mitral valve abnormalities and joint contractures were over-represented ($p<0.0001$). Also, an over-representation of missense mutations and an under-representation of non sense mutations were noted ($p<0.0001$). When considering the overall results of the genotype-phenotype correlation study in patients with a *FBN1* mutation, it appears that the location of mutations within exons 24-32 is the main severity criterion for Marfan syndrome.

P0114. Expanding the clinical spectrum of MYCN related Feingold syndrome

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Feingold syndrome (MIM#164280) is an autosomal dominant disorder characterized by variable combinations of microcephaly, limb malformations, oesophageal and duodenal atresias and learning disability/mental retardation. Hand and foot abnormalities may include hypoplastic thumbs, clinodactyly of second and fifth fingers, syndactyly (characteristically between second-third and fourth-fifth

toes), and shortened or absent middle phalanges. Cardiac and renal malformations, vertebral anomalies and deafness have also been described in a minority of patients. As a result of the versatile clinical picture, this entity has also been reported as ODED (oculo-duodeno-esophageal-digital) syndrome, microcephaly-oculo-digito-esophageal-duodenal syndrome and MMT (microcephaly-mesobrachyphalangy-tracheoesophageal-fistula) syndrome. However, some of the variable features of Feingold syndrome are included in "microcephaly-digital abnormalities-normal intelligence" (MIM#602585), described as an independent, distinct condition. The critical region of Feingold syndrome was mapped to chromosome 2p23-p24 (Celli et al., 2003) and a recent article revealed *MYCN* (2p24.1), as a causative gene in Feingold syndrome (Bokhoven et al., 2005). We report on two unrelated patients - expressing variable features of Feingold syndrome - who carry novel mutation of *MYCN*. One of their mothers carries the pathogenic mutation, but only possesses the clinical phenotype of "microcephaly-digital abnormalities-normal intelligence" disorder. Our cases highlight the significantly variable expressivity of *MYCN* mutations in Feingold syndrome and support evidence that "microcephaly-digital abnormalities-normal intelligence" represents a mild form of this genetic entity.

P0115. Fibrodysplasia Ossificans Progressiva: a case with calcification of papillary muscle of left ventricle.

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Fibrodysplasia ossificans progressiva (FOP) is a rare connective tissue disorder which is associated with fibroblast proliferation and extensive inflammatory infiltrates in the subcutaneous tissue, tendons, ligaments and muscles leading to heterotopic bone formation with progressive endochondral ossification. Major clinical findings are short great toe with hallux valgus, tender soft tissue lumps on trunk or extremities, progressive stiffness of neck, back and decreased mobility, occasionally deafness develop in the course of the disorder. We present a case of FOP in a girl with typical clinical and radiological findings. Interesingly, our case had calcification in the papillary muscle of the left ventricule which had not been reported before in the literature.

A 3-years-old girl was admitted to our hospital because of multiple hard lumps located on the scalp, neck, back and arms. She was the only child of a nonconsanguineous healthy couple and psychomotor development had been normal till now. On her physical examination, there were multiple painful lumps on her scalp, neck, extremities and paraspinal muscles of the back. These lumps were limiting the mobility of the neck, shoulder, elbow and the back and she had torticollis. She could not be able to sit or squat down by herself. She had bilateral short great toes with hallux valgus, inverted nipples and III/IV^{o} pan-systolic murmur. On her radiological examination, multiple amorphous calcifications located in the muscles were seen. Echocardiographic findings were influential as there was a calcification like echodense appearance in the middle part of the left ventricule papillary muscle.

P0116. A new fragile site at chromosome 18q22.2, possibly associated with in vivo chromosome breakage

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We are studying a patient with an 18q22.2 truncation, suffering from Beckwith-Wiedemann syndrome (BWS), characterized by overgrowth and loss of IGF2 imprinting. The father of the patient expressed a hitherto undescribed aphidicolin induced fragile site, located in the chromosomal breakpoint region of the infant. The chromosomal breakpoint was cloned and it was shown that the truncation is stabilized in vivo by the addition of repetitive telomeric sequence (TTAGGG). The breakpoint disrupts the *DOK6* gene, which plays an important role in the activation of receptor tyrosine kinases.

In order to investigate the potential role of the observed fragile site in the breakage mechanism we performed flexibility analysis, using the Twistflex computer program, of the entire 18q22.2 region. The results revealed that the 100 kb surrounding the breakpoint is extremely rich in AT-dinucleotide repeats, characteristic of aphidicolin induced fragile sites. Together, these data suggest that the breakpoint occurred within an aphidicolin sensitive fragile site, expressed in the father. It is the first time that an aphidicolin induced fragile site may be linked to in vivo chromosome breakage in the progeny.

Further study is necessary to investigate whether disturbances in this cascade could possibly be responsible for the clinical symptoms of the Beckwith Wiedemann syndrome.

P0117. Population screening for Fragile X syndrome in children using antiFMRP test

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Fragile X Syndrome (FXS) is a common genetic cause of mental retardation. Clinical picture is very mild in children. Our previous studies revealed a relatively low frequency of FXS in Romanian population. For this reason and because DNA tests are not yet available on a large scale we have introduced a population screening using antiFMRP immunohistochemical test. We have examined 254 children with delayed speech/ MR/ autism/ family history of MR or autism. We have applied a diagnostic score for children and selected 103 cases that were tested with antiFMRP test (done both on hair root and blood cells). Following the identification of the affected children, we have examined their families to identify new cases. Finally, 15 affected individuals were identified. Results in both immunohistochemical methods were concordant. We present the clinical features of these cases, as well as a statistical analysis of their frequency. The occurrence of different clinical and behavioral features at different ages will be provided. Based on our cases, the diagnostic score for children will be analyzed. We found that the method on hair root is less traumatic for the patient and we were able to reduce the amount of necessary reagents (15 times), reason why we decided to use this technique for future practice.

In conclusion we present a population screening using antiFMRP test in order to discuss the importance of different clinical features for diagnosis in children, as well as the methods we have used in order to reduce the cost of the test.

P0118. Fragile X screening in mental retardation

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The fragile X syndrome is the most frequent X linked Mental Retardation (XLMR) cause. It is due to mutation of FMR1 gene on Xq27.3 which consists of an expansion of triplets CGG located at exon 1. This mutation incidence is estimated to 1/4000 men.

Our study consist of the screening of 40 males having mental retardation associated to variable other clinical features. These patients consulted our service of Cytogenetic and Biology of the Reproduction from July 2004 to July 2005. The clinical investigation of these patients reveals no prenatal, no neonatal and no postnatal origin of their mental retardation. The caryotype was normal for all.

The molecular study, according to the international recommendations for the diagnosis of the fragile X syndrome, was performed by two different techniques: fluorescent CG rich PCR and Southern blot.

Four of the screened patients (10%) showed an expansion of their triplets CGG region confirming their X fragile status.

Among these 4 patients only one is 13 years old. This one presents strongly evocative clinical features of fragile X syndrome. The 3 others, aged between 6 and 9 years, have poor clinical features. Indeed, except MR, the other clinical criteria of X fragile syndrome appear only after puberty, which makes difficult the clinical diagnosis at a very young age.

Because of the important frequency of this syndrome in MR, its phenotypic heterogeneity and its great risk of recurrence in the same family, make its molecular diagnosis obligatory in front of any syndromic or not syndromic MR.

P0119. Fraser syndrome; Report of two affected siblings with a new Finding, in a consanguineous Iranian family

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Background: Fraser syndrome is a very rare autosomal recessive disorder, characterized by major malformations such as cryptophthalmos or anophthalmia, laryngeal abnormalities, orofacial clefting, craniofacial dysmorphism, genitourinary tract anomalies, mental retardation and musculoskletal anomalies.

Materials & Methods: Here, we report the clinical and pathological findings of two affected patients from an Iranian family. We knew the history of the first sib, so in the second pregnancy, we followed the fetus by ultrasound, and on the weeks 25, the same anomalies were detected in the fetus. The pregnancy was terminated and fetus extracted by hystrotomy.

Results: The clinical findings on this case were similar to the first sib. The fetus was sent for karyotyping and complete postmortem study. Extra findings in autopsy were: both lungs composed of two lobes (this is a new finding in this syndrome). Esophagus was narrow in its proximal part. Bilateral renal agenesis, small and hypo plastic bladder, and agenesis of internal genitalia were detected.

The blood and skin biopsy samples were sent for mutation analysis on FRAS I and FREM II genes. Now, we are waiting for the result of molecular analysis which is underway.

Conclusion: We should consider this rare syndrome in any fetus with IUFD and stillborn babies with these complex anomalies. High resolution ultrasound is a very efficient tool for detection of affected fetuses.

P0120. Fragile X syndrome-two complicated familal cases.

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Fragile X syndrome is the most common cause of inherited mental retardation caused by expansion of CGG repeats in *FMR1* gene- in full mutation number of repeats exceeds 200. Fragile X permutations (55-200 CGG repeats) occur more frequently in women having premature ovarian failure (POF).

We present data of two complicated familial fragile X syndrome cases.

Molecular studies were performed using fluoresence PCR and the CGG repeats lengths were measured by ABI PRISM 377 and/or ABI 310.

Family I. 7-years old boy was consulted and investigated due to mental retardation and behavioural problems. Family history was complicated with POF and mental retardation. As patient's mother had the history of POF, *in vitro* fertilization was performed, in which her sister's ovum was used. Molecular diagnostics of the patient revealed a full mutation (300 CGG repeats). Mother carried premutation with CGG repeats in one allele 77-79. Other family members were not investigated.

Family II. 3-years old boy was counselled due to mental retardation and autism. DNA diagnostics revealed that the patient had an unstable allele in premutation range (114 repeats) and the full mutation range (500-700 repeats). The patient's mother had normal allele (30 repeats) and other allele with CGG repeats in the premutation (83-134 triplets) and the full mutation range (400-550 triplets).

Conclusion. Detection of CGG repeats in *FMR1* among the women of reproductive age and in mentally retarded boys with a positive family history has a critical value in genetic counselling and prognosis in the family.

P0121. Mutation Detection of GALT gene in Iranian Galactosemia Patients

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One hundred and fifty unrelated families, clinically suspected of galactosemia were screened by qualitative measurement of galactose 1 phosphate uridyl transferase (GALT) activity in blood RBCs using Beutler's method. Deficient enzyme activity was confirmed in 18 families. All of these 18 families were submitted to the diagnosis of common mutations in GALT gene including Q188R, K285N using PCR-RFLP method. In order to determine the unknown mutations the entire coding region of GALT was subjected to sequencing. The most common molecular defect observed in Iranian population was Q188R (60%). Four rare mutations including S135L, K285N, A320T and Y209S were also detected.

P0122. Mutation detection for Glucocerebrosidase gene in an Iranian family with history of type 3 Gaucher disease

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Gaucher's disease is one of the most prevalent lysosomal storage disorders and a rare genetic disease. Partial deficiency of acid β-glucosidase is associated with parenchymal disease of the liver, spleen, and bone marrow with concomitant anemia and thrombocytopenia in non-neuronopathic, type 1 GD. Severe deficiency caused by severe mutations is additionally associated with neurological manifestations in the less common type 2 and type 3 GD subtypes.

Amongst the mutations characterized in patients presenting with GD, only three have a high frequency. The mutations c. 1226A>G (N370S), c.1448T>C (L444P), and 84insG (84G>GG) account for 55, 20, and 8% of cases, of the alleles evidenced in GD, respectively.

A family who had history of two affected sons with type 3 B GD were referred to our center for prenatal diagnosis. The disease emerges in early childhood, with predominantly visceral manifestations and severity of central nervous system lesions, progressing rapidly and resulting in death at 18 months of age and 2.5 years old respectively, due to the complications of portal or pulmonary hypertension. The neurological involvement was essentially restricted to horizontal supranuclear gaze palsy. After genetic counseling, direct DNA sequencing was performed for the couple who were second cousins. The results indicate that both parents are heterozygous for L444P mutation. Prenatal diagnosis showed that the fetus was also heterozygous for this mutation and not affected with GD.

It appears that c.1448T>C (L444P) mutation is one of the common mutations which could be noticeable for molecular diagnosis of GD in Iranian population.

P0123. Novel missense mutation in *M1S1* gene in Iranian Gelatinous Drop-Like Corneal Dystrophy (GDLD) patients

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M1S1 (membrane component, chromosome 1, surface marker 1) has been identified as a gene responsible for GDLD in a study on Japanese patients. The M1S1 protein is a membrane cell surface glycoprotein expressed in the cornea, multistratified epithelia, trophoblasts and most carcinomas. The physiological function of M1S1 is not well understood, but it be involved in the establishment of intercellular connections. Not all GDLD patients carry mutations in *M1S1*. Disease causing mutations found in *M1S1* are usually nonsense or frameshift mutations, and missense mutations are relatively rare. *M1S1* (NT_032977) was screened in the proband of three Iranian familial cases of GDLD. The patients were offsprings of consanguineous marriages and from three distinct region of Iran. A novel homozygous putative disease causing missense mutation, N.679G>A (p.E227K) was identified by sequencing. The mutated alleles were also homozygous for two polymorphisms, suggesting identity by decent. The mutated nucleotide was not found by RFLP among 60 ethnically matched control individuals. The proband of the two additional Iranian families were also found to carry the same mutation in the homozygous state.

We are now establishing whether the chromosomes of all the patients share the same SNP haplotype.

P0124. A new case of geleophysic dwarphism - clinical presentation

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We presente the case of KR patient, 1 year 4 months old. She is was the first child of unrelated, healthy and young parents. The pregnancy has a normal evolution.
She was born at term by normal delivery, weighing 2100 g.
At the clinical examination we can observe a disproportionate short stature with short limbs, typical dysmorphic face with “happy” facial appearance (round face, thin lips, micro-retrognatia) cardiac anomalies with atrial septal defect, left hepatic lobe enlargement without signs of storage disease, mild developmental delay. Based on the clinical exam we establish the diagnosis of geleophysic dwarphism.
Geleophysic dwarphism is a rare, autosomal recessive disorder with disproportionate short stature, characteristic facial appearance which is described as “happy” and survival to adulthood. In our case the diagnosis was not establish until the patient was 1 year and 4 months old.

P0125. AZF deletions, MTHFR C677T genepolymorphism and OCTN2 mutation analysis in male infertility

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Objectives Microdeletions of azoospermia factor AZF a, b, c region and MTHFR C677T polymorphism can be responsible for male infertility. Despite decreased carnitine level in azoospermic seminal fluid, the role of carnitine tarnsporter function has not been studied.
Aim: To study the frequency of AZF deletions, MTHFR C677T genepolymorphism and OCTN2 mutations in Hungarian infertile males.
Materials and Methods Y chromosome microdeletion screening of most frequently deleted regions such as sY 254, 86, 127, 84, 134, and 255 was studied by multiplex PCR after isolation of genomic DNA from 280 azoo- or oligozoospermic males. MTHFR C677T mutation was determined by PCR. The ten exons of the SLC22A4 gene encoding the OCTN1 carnitine transporter were sequenced by intron based primers in 20 azoospermic patients.
Results AZF microdeletions were found in 2 of 280 patients. The prevalence of C/C, C/T and T/T polymorphism was 43.1%, 39.5% and 17.3% in the infertility group and 38.4%, 53% and 8.6% in the control population, respectively. No pathology associated mutations in the SLC22A4 gene could be detected..
Conclusions The low prevalence (0.7%) of AZF deletions is surprising. The MTHFR polymorphism might have a role in the pathogenesis of male infertility. No evidence was found that the abnormalities of the OCTN2 can have role in azoospermia.

P0126. Gerodermia osteodysplastica: report of three boys in a family

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Three boys (3 yr, 2 yr and 8 mo) who were second cousins with each other were referred to our department because of atypical facies and delayed motor development. Gerodermia osteodysplastica was diagnosed in these cases who had the distinct facial apperance with sagging cheecks, thin hair, large fontanelles, wrinkled and lax skin on the dorsum on the hands and feet, visually prominent veins and hypermobility in the metacarpophalangeal joints. Radiological investigations showed generalised mild osteoporosis, vertebral compression, numerous wormian bones in the lambdoid sutures. Mandibular prognathism was described in case 1 and 2 , unilateral hip dislocation was described in case 1 and inguinal hernia was described in case 3. Gerodermia osteodysplastica is a rare autosomal recessive disorder characterized by wrinkled and lax skin with reduced elasticity mainly on the dorsum of the hands and feet, aged appearence, hyperextensible joints and osteoporosis. Intra and interfamilial variabilty in the severity of the condition has been noted, especially with respect to the susceptibility to fractures and alternations in stature. It has been suggested that Gerodermia osteodysplastica and Wrinkly Skin syndrome could represent variable manifestations of the same disorder. The presence of intrauterine and postnatal growth retardation in Wrinkly Skin syndrome and the presence of osteoporosis and other radiological findings in Gerodermia osteodysplastica is helpful in differential diagnosis. In conclucion, Gerodermia osteodysplastica should be considered in a patient with cutis laxa accompanied by visually prominent veins, osteoporosis and joint laxity.

P0127. A novel microdeletion in p34.2p34.3 of chromosome 1 involving the *SLC2A1 (GLUT1)* gene in a boy with severe mental retardation.

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A *de novo* 4.1 Mb microdeletion of chromosome 1p34.2p34.3 identified by genome wide array-based comparative genomic hybridisation (array-CGH) is reported in a boy with severe mental retardation, microcephaly, pronounced hypotonia and facial dysmorphism. The deleted region encompasses 48 genes among which the Glucose transporter 1 (*SLC2A1 or GLUT1*) gene. The deletion of the GLUT1 gene was in line with the abnormal cerebrospinal fluid/blood glucose ratio indicative of GLUT1 deficiency syndrome (MIM 606777). In addition the deletion was confirmed by region specific Multiplex Ligation-dependent Probe Amplification (MLPA). GLUT1 deficiency syndrome is clinically characterised by intractable seizures, developmental delay, acquired microcephaly, and a complex motor disorder. A ketogenic diet is a rational and highly effective treatment, especially with regard to seizure control. This report illustrates that identifying a microdeletion as the cause of mental retardation is not only important for genetic counseling but also may lead to therapeutic intervention.

P0128. Simultaneous detection of copy number changes and CpG methylation of the differentially imprinted GNAS complex locus using Methylation-specific MLPA

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GNAS is a complex gene with multiple imprinted promoters located on chromosome 20q13. Epigenetic defects in the imprinted GNAS cluster are associated with pseudohypoparathyroidism type 1b (PHP1b). The downstream promoter for the stimulatory G protein α-subunit is unmethylated in all tissues apart for the renal proximal tubules. The first and most upstream GNAS Differentially Methylated Region (DMR) generates the NESP55 transcript and is paternally methylated, the second DMR is maternally methylated and generates the XLαs and the NESP Antisense (AS) transcript. Finally, the third DMR generates the 1A transcript and is also maternally methylated. Here we describe a novel application of Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) which can detect changes in both CpG methylation of all GNAS DMRs as well as the copy number of the whole region. A quantitative methylation and copy number analysis has the advantage of detecting all of the major classes of molecular defects involved in PHP1b (deletions, uniparental disomy, and imprinting mutations) without the need for parental DNA. We successfully used MS-MLPA to detect copy number and methylation status of the genes in the chromosome 20q13 in 30 mutation negative DNA samples of patients referred to the clinic with a possible GNAS defect.

P0129. Goldenhar syndrome: clinical manifestations in Greek patients

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Goldenhar (GS) syndrome is a well-recognised developmental disorder involving first and second branchial arches and characterized by considerable phenotypic variability. The present study presents clinical data on the morphologic features, hearing, ophthalmologic, orthopaedic, neurological, cardiovascular, genitourinary and gastrointestinal evaluation of 17 Greek patients (one pair of monozygotic twins) aged 20 days to 23 years with the clinical diagnosis of GS and with a normal karyotype. The most consistent findings were auricular defects (94%), followed by facial (76%) and ocular anomalies (65%), 70% unilateral, mainly right-sided. In the majority of our patients (90%) mandibular hypoplasia was ipsilateral to the dysplastic ear or the most severely affected ear in bilateral cases. Hearing loss, mainly conductive, was noted in 76% of our patients. Skeletal defects were evident in 23%, while cardiovascular, genitourinary and gastrointestinal in 18%, 23% and 12% respectively. The most frequent neurological manifestation was facial nerve paralysis (12%), while the incidence of mental retardation was higher (23%) than shown in the literature, presumably attributed to the severe hearing and vision loss. In the pair of monozygotic twins of our study was noted discordance of clinical findings. Precise evaluation of GS patients and multidisciplinary care management is necessary to avoid possible complications of many systems and to offer appropriate genetic counselling to the family.

P0130. 31 cases with Goldenhar syndrome: clinical, audiological, cytogenetic and MR findings.

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The oculoauriculovertebral complex is phenotypically variable due to heterogenous causes. In this study 15 boys and 16 girls, totally 31 patients with Goldenhar syndrome who were followed at Hacettepe University Department of Clinical Genetics between 1968 and 2004 were reviewed. Characteristic findings which were preauricular skintag were found in 93% ; microtia in 60%, hemifasial microsomia in 70%; epibulbar dermoid in 39% and vertebral anomalies in 71% of patients. Cardiac malformations were evaluated in 29 cases. 11 cases of 29 had congenital heart disease. MR studies were performed in 19 cases. 9 of 19 cases were diagnosed with CNS malformations such as Arnold-Chiari type II, corpus callosum hypoplasia, etc. Audiologic evaluation was performed in 23 patients. 69 % of patients had hearing deficiency. Interestingly 3 patients were born from ICSI pregnancies.
Peripheral chromosome analyses were performed in 29 patients and only 1 of them had 47,XX,+der(22)t(11;22)(q23;q11). Interestingly this case had maternal balanced t(11;22) in four members of her family. This translocation was proven by FISH (fluorescence in situ hybridization) analysis. Additionally CATCH 22 deletion analysis was performed in 13 of 31 patients. All of them were CATCH 22 deletion negative.
In conclusion chromosomal studies should be performed in every case with Goldenhar syndrome for a possibility of chromosomal anomaly.

P0131. Gonadoblastoma in a 46,XY young woman with primary amenorrhea

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A 16-year old young woman presented with primary amenorrhea. Clinically, she had normal female external genitalia, no hirsutism and a height of 175 cm. Tanner stages were B3 and PH4 with elevated LH- and FSH-levels documenting hypergonadotropic hypogonadism. Chromosome analysis on peripheral blood cells showed a 46,XY karyotype (20 metaphases, GTG-banding, 500 bands, 2 independent samples) with typical fluorescence of the Y-chromosome in Q-banding. FISH-analysis using a wcp (Y)-probe resulted in whole chromosome painting of the Y-chromosome. The presence of the SRY-region (Yp11.2) was proven by FISH. Moreover, the Y-specific loci ZFY, SRY, AZFa, AZFb and AZFc were detectable by multiplex-PCR in 2 formats. Sequencing of the coding region of the androgen receptor gene did not detect a pathogenic mutation. On laparascopy, ovarian structures coexisted with testicular remnants and were completely removed. The karyotype of the gonadal fibroblasts was also 46, XY (10 metaphases). FISH-analysis with a sex chromosome specific probe (CEP 18/X/Y) showed an XY-genotype in 64 of 65 metaphases and 453 of 460 interphases. Histological examination of the gonads showed atypical immature testicular structures, fallopian tubes and a gonadoblastoma. Reviewing the literature, 46,XY individuals with partial or mixed gonadal dysgenesis are at a much higher risk for malignancy than individuals with numerical sex chromosome aberrations and / or individuals with pure gonadal dysgenesis. Our case illustrates that gonadoblastomas occur in asymptomatic individuals with gonadal dysgenesis emphazising the need for surgical removal of the gonads as soon as possible.

P0132. Gorlin syndrome - first DNA analysis in Czech republic. Clinical features and DNA analysis of seven patients.

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Gorlin syndrome - NBSSC is a rare, autosomal dominantly inherited pre-cancer syndrome affecting several body systems.
The main features are odontogenic keratocysts and multiple basal cell neavi, which have malignant potential.
Characteristic feature of the syndrome is a high predisposition to cancerous growth, mainly development of basal cell carcinomas and higher incidence of congenital birth defects.
Clinical diagnosis requires presence of at least two major signs such as odontogenic keratocysts, basal cell carcinomas, palmar pits and familial occurrence. The gene associated with the condition is a tumor supresor gene (PTCH), which maps to the 9q22,3-q31 region. The tumors associated with Gorlin syndrome are fibrosarcoma, rhabdomyosarcoma, meningioma, and meduloblastoma.
We present seven patients (six men and one woman) with clinical features of NBSSC syndrome, ascertained because of odontogenic cysts manifesting before 20 years of age. Five of these patients represent new mutations; mother and her proband daughter represent one familial case.
Mutation analysis of the coding region of the PTCH gene was performed in four unrelated patients and mutations were detected in all of them. All exons and exon-intron boundaries were amplified with PCR and both forward and reverse strand were sequenced with appropriate PCR-primers. The following mutations were identified:

1. c.585-3C>G
2. c.[915delC (+)3583A>T], p.[Ala306fs (+)Thr1195Ser]
3. c.1348-1G>A
4. c.2179delT, p.Cys727fs

All patients are followed up by dermatologists, dentists and oncologists and no malignancies were observed. The knowledge of the gene mutation enables us to offer prenatal diagnosis to the patients of fertile age.
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P0133. Two Novel Mutations in Iranian Haemophilia B Patients

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Background: Hemophilia B or Christmas disease is an inherited recessive X-linked bleeding disorder which results from deficiency or defect of procoagulant factor IX (FIX). The factor IX gene spans 35kb of DNA and comprises 8 exons, designated a-h (1-8). Factor IX mRNA is 2.8kb and encodes a mature protein of 415 amino acids.
Methods: Genomic DNA of 31 patients referred from Imam Khomeini and Omid hospitals were extracted according to standard protocols. PCR amplification and SSCP on nondenaturing polyacrylamid gel, were performed on each sample for eight exons separately. The result of SSCP for each sample was compared to normal ones and sequencings were performed for those with different migration patterns.
Results and conclusion: The sequencing results showed 70.8% missense mutation, 16.7% deletion, 8.3% nonsense mutation and

4.2% insertion. This was similar to reports haemophilia B mutation database. In this study SSCP had 77.5% sensitivity. Failure of PCR amplification of a patient led us to recognize a large deletion (g & h exons). In addition we found two novel mutations in two unrelated patients. C 6364 T and A 17690 C changes were found in Hb004 and HB078 respectively. These represented R -4 W and N 92 T. These novel mutations occurred in one of critical regions of CpG. Parallel to our expectation these patients suffered from severe disease.

P0134. Role of mtDNA in Iranian patients with Hypertrophic Cardiomyopathy

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Hypertrophic cardiomyopathy is characterized by hypertrophy of ventricles and intrventricular septum. Patients could develop serious complications including heart failure, arrhythmias and sudden death. The disorder has been estimated to occur in 0.05%-0.2 percentage of population. Recently mitochondrial DNA mutations have been associated with cardiomyopathies. Mitochondria are the major site of energy production in the cell. Thus, it is reasonable to assume that energy dependant tissues such as heart, affected by mitochondrial dysfunction. Mitochondrial (mt) DNA mutations are hypothesized to be involved in the pathogenesis of Hypertrophic Cardiomyopathy, because the mtDNA encodes 13 polypeptides that are essential for oxidative phosphorylation, upon which the heart relies for energy. In this study, 31 Iranian hypertrophic cardiomyopathy patients for mitochondrial DNA point mutations and deletions were screened. Results: some mutations in G3338A (Val>Met), G9053A (Ser>Asn), G9055A (Ala>Thr), T3285C in tRNA Leucine, were detected, and also26 polymorphism were found that before were reported, 15 polymorphism that were not reported. Three different deletions were found in seven patients (13.46%). Three patients (5.76%) showed 8.6 kb deletion. Three patients (5.76%) had a 7.4 kb deletion. One patient (1.92%) had 4977 bp “common deletion

P0135. Genetic etiology and spectrum of mutations in GJB2 gene in 111 families investigated in our hereditary hearing impairment clinic

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Hereditary hearing impairment clinic was established at our institute in October 2003. Until the present 111 families have been investigated. Genetic etiology of hearing impairment was found at 71.2 % of families - 11.7 % with autosomal dominant inheritance, 40.6% with autosomal recessive inheritance and 8.1% with genetic syndromes or another genetic disease with deafness. At 10.8 % families the mode of inheritance could not be determined. The rest (28.8%) were families with idiopathic hearing loss.

The mutations in the GJB2 gene (Cx26) were investigated at 139 patients from 78 families 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 3 mutations not reported before (Ala149Thr, Ile140Ser, c.683+3 C to A). At least one pathogenic mutation was found at 70 (50.4%) patients. Both pathogenic mutations were detected at 29 (20.9%) patients. No pathogenic GJB2 mutation was detected at 53 (38.1%) patients and 16 (11.5%) patients are carriers of various polymorphisms (Met34Thr, Val153Ile, Arg127His, Phe83Leu etc.). The mutation 35delG accounts for 80.8% of detected disease mutations. Mutations 313del14 and IVS 1+1 G to A (-3170 G to A) in the non-coding region account each for 7.1% of detected disease mutations.

P0136. Accessing genetical and environmental factors of hearing loss in 354 families in Iran (Qom and Markazi provinces)

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Introduction: Hearing loss (HL) is the most prevalent sensorineural defect in humans. Mild to severe and profound HL occurs in about l.0 per 1000 births. Many previous studies have shown that about 50% of deafness is genetic and 50% is environmental or of unknown origin.
Materials and Methods: Questionaires were distributed in deaf schools, hearing loss centers and rehabilitation centers in Qom and Markazi provinces and were collected after filling by the parents.The information such as age, sex, number of deaf individuals in families and their close relatives, the marriage type of the parents and etiology were extracted from questionnaires and were analysed by SPSS software .
Result: 354 Questionaires containing complete information from hearing loss families were collected. 59.3% and 36.7% of parents have consanguineous and nonconsanguineous marriage ,respectively and the marriage type of 4% were not determined. By assesing the filled questionnaires and pedigrees, the deafness aetiology in the studies population was categorized in genetic (70.9 %), environment (9%) and unknown (20.1%).
Conclusion: Genetic factor with autosomal recessive inheritance pattern was the most important cause of hearing loss due to the high prevalence of consanguineous marriage. This increased genetic factors to more than 50%. Large family sizes also increase the frequency of hearing loss in families that have deaf with genetical background of this disorder. In this study, environmental and unknown factors were second cause of HL. We can interestingly reduce frequency of HL in Iran by discouraging consanguineous marriage, health education, population regeneration control especially for high risk families.

P0137. A new syndrome of microtia with mixed tyoe hearing loss, renal agenesis, and multiple skeletal anomalies

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We report on a 17-year man presented with unreported combinations of right sided microtia and preauricular skin tag with conductive type hearing loss, unilateral renal agenesis, partial syndactyly of 4th and 5th metacarpals, multiple tarsal coalitions, absent toe, and hypoplastic tibia and fibula. Radiological and clinical findings did not match with the previously described syndromes with respect to the type of anomalies seen in the case. Chromosomal analysis from peripheral blood samples and skin from the index case was performed. Five hundred fifty banding was applied to metaphases cultured from both the skin and peripheral blood samples and all family members showed a normal karyotype. Sequence analysis was performed for exon 2 of SALL1, but mutations could not be detected. We propose that this is a new syndrome.

P0138. A rare manifestation of hereditary haemochromatosis in association with anaemia in two young women

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Hereditary haemochromatosis (HH) is a frequently inherited AR metabolic disorder in the European population. HH causes iron accumulation in patients liver, pancreas, and other internal organs. HH in young women is rearly reported, because of regular loss of stored iron by menstrual blood. Here we report two cases of HH manifestation in young women.
Case 1: age 24, referred to hepathologist because of icterus. All viral markers were negative, liver biopsy showed cirrhosis, in blood biochemistry high serum ferritin (SF) was noted (1225 µg/l). Her DNA was checked for mutations in HFE gene. The genotype revealed was C282Y/H63D. Unfortunately the phlebotomy treatment is not possible because she has anaemia (Hb 106 g/l). Nevertheless SF decreased spontaneously to 835 µg/l, but later increased to 1300 µg/l.
Case 2: age 35, presented with chronic fatigue 8 months after hysterectomy due to myomatosis. Investigation revealed anaemia (Hb 109 g/l), elevated SF 403 µg/l, which rised suspection for the HH. The HFE analysis showed genotype C282Y/C282Y. Because of her low Hb, she initiated treatment with desferal, but that was poorly tolerated,

because of side effects.
Although case 2 has explanation for iron overload (hysterectomy), the cause of anaemia has not been found, though both women consulted a haemothologist. As there have been some reports of digenic inheritance of HH, we have done sequencing analysis of juvenile haemochromatosis gene HAMP and checked TFR2 mutation Y250, but no mutations were found. The explanation of such HH manifestation in young women still needs a further investigation.

P0139. MLPA-based screening for disease-causing copy number alterations in the hereditary spastic paraplegia genes *SPG3A* and *SPG4*

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The hereditary spastic paraplegias (HSPs) are neuro-degenerative disorders associated with progressive spastic weakness of the lower limbs. Despite 30 different disease loci reported to date, mutations in either *SPG3A* or *SPG4* are found in 35-50% of cases by conventional sequencing. Additional pedigrees, linking to one of these loci but apparently being mutation negative, may inherit larger genomic aberrations. We have developed a multiplex ligation-dependent probe amplification (MLPA) assay allowing simultaneous standardised copy-number screening of almost every *SPG3A* and *SPG4* exon. In a cohort of 18 HSP families, we identified 3 aberrant MLPA profiles segregating with the disease and exclusively affecting *SPG4*-specific probes. Long-range PCR followed by sequencing revealed two novel multi-exonic deletions and a previously reported 3bp deletion as underlying these MLPA results. Breakpoint analysis suggested specific but differing mutational events as having resulted in the deletions. Investigation at the cDNA level showed that certain deletions may negatively influence stability of the transcript from the mutated *SPG4* allele. According to these preliminary results, partial gene deletions in *SPG4* seem to be a rather frequent, but private, mutation type causing HSP. To obtain more solid data on their prevalence, we are currently investigating a larger series of index cases. The MLPA kit described here may, eventually, become a useful tool in the molecular diagnosis of HSP.
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P0140. Heterotaxy in a family with autosomal dominant inheritance.

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Familial heterotaxy is genetically heterogeneous and may be inherited as an autosomal dominant, autosomal recessive or an X-linked trait. The nomenclature of situs abnormalities is somewhat confusing, but most authors define heterotaxy as any deviation from the normal situs solitus. The phenotype within a family with heterotaxy has been reported to vary. The present study comprises a family with four affected individuals in two generations, including male to male transition. Three brothers all had various degrees of heterotaxy, including complete situs inversus, transposition, and situs ambiguus, respectively. The fourth brother is phenotypically normal. One of the affected brothers and his wife terminated a pregnancy revealing a male fetus with L-transposition. Autosomal dominant inheritance with reduced penetrance is suggested as are the parents have no clinical signs of left-right malformations.
To date, relatively few genes have been implicated in situs disorders, including *CFC1 (CRYPTIC)*, *ZIC3, LEFTYA, ACVR2B, CRELD1* and *NKX2.5*. To investigate whether mutations in any of these genes could be detected in the present family, DNA was extracted from blood from the patient with situs inversus totalis. Sequencing of *CFC1*, *ZIC3, LEFTYA, CRELD1* and *NKX2.5* revealed no mutations. Further analysis of *ACVR2B* is ongoing.

P0141. Lobar Holoprosencephaly - positive diagnosis

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Introduction: Holoprosencephaly appears between the 4-th to 8-th week of pregnancy due to the lack of cleavage of the prosencephalus in the telencephalus and diencephalus,. The attendance is 1 at 10.000 live newborn,; 60 times higher at aborted human embryos. Depending on the degree of differentiation and severity we can have 3 subtypes of holoprosencephaly: alobar. semilobar and lobar.
Material and Method: The authors present a study on 3 premature newborns, with lobar holoprosencephaly, an extremely rare affection in the current medical practice. Two of the patients didn't show any symptoms until the age of two weeks when they presented generalized tonic-clonic seizure. One of the newborns showed other associated malformations: unilateral anophthalmia and congenital septal defect. Cerebral lesions were highlighted by cerebral imaging methods - head ultrasonography, CT in one of the cases and NMR in 2 of the cases. Those investigations showed specific lesions at the median line. The karyotype was normal in 2 of the cases; one of the cases presented trisomy 13. The evolution of the cases was severe, causing death in one of the cases and slowly evoluting with recurrent seizures and motor- and psychic retardation in two of the cases.
Conclusions: Lobar holoprosencephaly is a rare affection, without specific clinical expression and its diagnosis can be easily missed in the neonatal period. Cerebral imaging was the primary method in the setting of the diagnosis: head ultrasonography tracked down the lesions and CT and NMR established their extension.

P0142. SIX3, ZIC2 and SHH mutations in a series of holoprosencephaly patients.

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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 analysis. A series of in total 47 sporadic and familial HPE cases with a variable clinical spectrum has been analysed. We detected 10 pathogenic mutations (21%), 5 out of 40 sporadic cases (13%) and 5 out of 7 familial cases (71%). One of the familial cases was caused by a mutation in parental germ cells. Four mutations were detected in the SIX3 gene, four mutations in the ZIC2 gene and two mutations in the SHH gene. 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. This illustrates the importance of further identification of new genes.

P0143. Two cases with progressive movement disorder: from undiagnosed condition to confirming juvenile form of Huntington's disease

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Huntington disease (HD) is a fatal autosomal dominant neurodegenerative disorder caused by high instability and extension of CAG sequences within the coding region of IT-15 gene. Juvenile-onset HD before the age 20 years occurs in about 5% of HD cases, and is associated with very large (more than 60) CAG repeat expansions. Juvenile HD manifested mostly as an ataxic syndrome with bradykinesia, rigidity, epileptic seizures, and dystonia whereas adult onset is more a prominent involuntary movement disorder with personality changes, and dementia.
We present clinical features and results of DNA analysis in 2 patients. Patient 1 was a 13-year-old girl presented with a 6 year history of declining school performance, loss of coordination, rigidity, impaired speech. This patient had cleft lip and clinical diagnosis of myotonic

dystrophy until she was referred to the Medical Genetics Centre (MGC). Molecular genetic analysis of genomic DNA revealed that patient had one normal-sized allele and one abnormally expanded allele with 90 CAG repeats.
Patient 2 was a 13-year-old boy. His developmental milestones were abnormal and at age 11 years diagnosed autism. By 12 years of age he started having difficulty speech impairment, loss of coordination, rigidity. He had been diagnosed with Wilson disease by clinical features. Molecular genetic analysis in MGC revealed that patient had one normal-sized allele and one abnormally expanded allele with 85 CAG repeats. Both patients had a positive paternal family history of HD.
Conclusion: juvenile HD should be considered in children suffering from a progressive movement disorder.

P0144. Molecular testing for Huntington disease in Poland: 10 years of experience

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Huntington disease (HD) belongs to neurodegenerative disorders resulting from polyglutamine coding CAG repeat expansion in the *IT15* locus on chromosome 4p. It is an autosomal dominant, progressive and late onset, disorder characterised by involuntary choreatic movements, cognition and emotion abnormalities. The anticipation phenomenon and strong negative correlation between age at onset and CAG repeats number is observed like in other polyglutaminopathies.
Molecular diagnostics of HD has been carried on in the Department of Genetics in the Institute of Psychiatry and Neurology since 1995. During 10 years we have performed molecular analysis in 1450 patients suspected of HD. Genetic analyses - symptomatic and predictive testing - confirmed dynamic mutation in locus *IT15* in 830 individuals.
Among those subjects 670 were manifesting symptoms of HD and 165 were in preclinical stage of the disease; in seven cases prenatal tests were performed. Affected subjects were grouped in 600 pedigrees. Moreover, in two pedigrees, we documented two de novo mutations during paternal transmission. Our genetic tests revealed also 106 individuals with more than 50 CAG repeats. Among them there were 39 juvenile cases of the age at onset below 20 years (range 2-19). As for alleles of incomplete penetrance (36-39 $(CAG)_n$) we have detected 27 cases; we came across one contraction phenomenon which occurred during maternal transmission.

P0145. Vitamin D dependent Rickets Type II; Report of Two Affected Siblings in a Consanguineous Iranian family and Review of the Literature

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Hereditary vitamin D resistant rickets is a genetically determined and rare autosomal recessive disorder, most often caused by mutations in the VitD receptor gene. It usually presents with rachitic changes not responsive to VitD. Circulating levels of 1,25(OH)2VitD3 is elevated, thus differentiating it from VitD dependent rickets type I. Alopecia of the scalp or whole body is seen in some families with type II variant. This is usually associated with a more sever phenotype.
In this report, we present our findings on a family exhibited the typical clinical features of HVDRR in two siblings. The proband is now an 18-month-old boy. He is the third offspring of a healthy couple. At the end of the first month of his life alopecia occurred and progressed to total loss of his scalp hair, along with refractory rickets.
Cardinal findings in our case were: alopecia totalis, renal tubular acidosis, mild generalized aminoaciduria, refractory rickets, high alkaline phosphatase, and hyperparathyroidism. Skin biopsy performed and the result was alopecia areata. Investigation for detection of molecular abnormality is underway.
The older child of the family was a boy which had similar disease and died because of its complications at the age of 32 month. The 2nd child is a healthy 5-year-old girl. Parents are relatives. We should be aware of this very rare disease, whenever we see a patient is suffering from refractory rickets with alopecia.

P0146. Congenital hydrocephaly-ultrasound diagnosis

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Diagnostical framing of the echography find out disease, correlation between the imaging and clinical marks, setting of the evolutional stage and therapeutical indication.
Material and Method: The study contained 34 cases of ventriculomegaly, selected by clinical and imaging criteria from the premature newborn hospitalized in the Clinic of Neonatology. Head ultrasonography was used as method of diagnosis and prognosis evaluation.
Results: Hydrocephaly was associated with: meningomyelocele in 8 of the cases, meningoencephalocele in 3 of the cases, Dandy-Walker malformation in 4 of the cases, agenesis of the corpus callosus in 5 of the cases, malformation of the Galen's vein in 2 of the cases, arachnoid kyst in 4 of the cases, lobar holoprosencephaly in 3 of the cases. In 4 of the cause couldn't be found at they didn't present any associated malformations. 5 of the cases from those with cranio-vertebral dysraphism presented Arnold/Chiari II malformations associated with meningomyelocele and 3 of those cases presented Arnold/Chiari III malformation associated with meningoencephalocele . The clinical manifestations were those of the classic hydrocephaly associated with: recurring seizures, paresis, paralysis of the inferior limbs in 4 cases, apnea crisis, acrocyanosis. Cerebral imaging was used to appreciate the ventricular enlargement and the degree of compression of the cerebral tissue. The evolution of the cases showed a high rate of decease- 51,4 %.*Conclusions:* The fast evoluting hydrocephaly was the primary cause of death at the cases with associated malformations. The most frequently met malformation types were: cranio- cerebral dysraphism and agenesis of the corpus callosum.

P0147. Mutation spectra of *ABCC8* gene in Spanish patients with Hyperinsulinism of Infancy (HI)

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Hyperinsulinism of Infancy (HI; OMIM: 256450) is a clinical disorder characterized by deregulation of insulin secretion that leads to profound hypoglycemia. Mutations in genes encoding the ATP-regulated potassium channels of the pancreatic ß-cell, namely ABCC8 (SUR1) and KCNJ11 (Kir6.2), are the major genetic known cause of the disease. To elucidate the genetic etiology of HI in the uncharacterized Spanish population, we conducted extensive sequencing analysis of the ABCC8 (83.5Kb) and KCNJ11 (1.7Kb) genes in 34 Spanish HI patients. Mutations in ABCC8 were detected for both alleles in 13 patients, while ten patients carried only one mutation in one of the ABCC8 alleles. We have detected 22 novel and seven previously described mutations in ABCC8, ~60% of them lead to a premature termination signal, which would result in truncated SUR1 proteins. No mutations were found in the KCNJ11 gene. In addition, we report for the first time a 3914bp macrodeletion associated with the HI disorder. The potential pathogenicity of several additional variants is discussed. The spatial pattern of three pathological mutations suggests possible geographical founder effects. This work reveals for first time the involvement of KATP channels in the pathogenesis of an important proportion (~68%) of Spanish HI patients. The spectrum of mutations in Spanish HI patients provides an important tool for diagnosis and prognosis of HI patients in the Spanish population, as well as for genetic counseling of HI families.

P0148. Molecular-genetic study *PTS* gene and *QDPR* gene of Russian PKU patients.

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Hyperphenylalaninemia (HPA) may be caused by deficiency of phenylalanine hydroxylase or tetrahydrobiopterin (BH4), the essential cofactor for the aromatic amino acid hydroxylases. The most frequent form of this cofactor deficiency is due to lack of 6-pyruvoyl-tetrahydropterin synthase (PTPS) activity and dihydropteridine reductase (DHPR) activity.
We report the molecular genetic study pyruvoyltetrahydropterin synthase (*PTS*) gene and dihydropteridine reductase (*QDPR*) gene in the group of 12 PKU-patients from different Russian regions without mutations in *PAH* gene. We have found that one patient from this group have novel mutation p.Ala135Asp in homozygous in the *QDPR* gene. Her mother has this mutation in heterozygous but father are inaccessible for investigation. Two known mutations (p.Asn72Lys and p.Thr106Met) were determined in compound heterozygosity in two unrelated patients from different Russian regions. Four new mutations were found in the *PTS* gene. One patient was compound heterozygosity with two missense mutations: p.Ser32Gly and p.Val59Gly. Another patient showed new mutation IVS5-1g>a in heterozygous state in compound with unidentified mutation. The substitution g.7068G>A in 9 bp after stop codon was determined in one patient in heterozygous state but none of 60 population donors reviled this alteration.
All patients have psychomotor system delay in spite of low phenylalanine serum level at low- phenylalanine diet therapy.

P0149. Novel Myosin Binding Protein C founder mutations may confer severe hypertrophic cardiomyopathy

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Background Familial hypertrophic cardiomyopathy (HCM) is caused by mutations in more than 11 genes, mostly encoding sarcomeric proteins. Most HCM mutations arise independently; founder effects are rarely observed. Mutations in the sarcomeric Myosin-Binding-Protein-C-gene (MYBPC3) are associated with late onset HCM and a relatively benign clinical expression. Previously we identified the MYBPC3 2373insG mutation as an important Dutch HCM founder mutation. The MYBPC3 gene was sequenced in 208 Dutch HCM probands to establish the prevalence of MYBPC3 associated HCM in the Dutch population.
Results A pathogenic MYBPC3-gene mutation was identified in 95/208 (46%) probands. In addition to the 2373insG mutation, found in 19% (38/208), we identified the R943X and 2864delCT mutations in 8% (16/208) and 7% (15/208) respectively. Subsequent extended haplotype analysis demonstrated the R943X and 2864delCT mutations to be additional HCM founder mutations in the Dutch population.
Cardiological parameters were analysed in 16 families (23 carriers) with the R943X mutation and in 12 families (20 carriers) with the 2864delCT mutation. Sudden death was observed in 56% (9/16) and 58% (7/12) of the respective families.
HCM prognosis was considered malignant in 38% (5/13) of the R943X families and in 56% (5/9) of the 2864delCT families.
Conclusion The R943X and 2864delCT mutations are additional MYBPC3 founder mutations in the Dutch population. Intrafamilial variability in age at onset and in severity of clinical symptoms was observed. In contrast to earlier studies reporting MYBPC3 mutations to be relatively benign, both HCM mutations are associated with severe heart failure symptoms and possible malignant prognosis.

P0150. Prevalence of Telethonin encoding T-cap gene in a consecutive series of 200 patients diagnosed with Hypertrophic (HCM) and Dilated cardiomyopathy (DCM)

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Telethonin is a sarcomeric protein of 19 kDa possibly localized to the Z-disc of adult striated skeletal and cardiac muscles, where it interacts with the protein titin. Mutations in the T-cap gene encoding telethonin cause LGMD2G, a relatively mild form of autosomal recessive LGMD. Mutations of the T-cap gene have been recently reported in autosomal dominant HCM and DCM without myopathy.
We aimed at determining the prevalence of T-cap gene mutations in a consecutive series of 200 patients clinically diagnosed with familial and sporadic DCM (n = 100), and HCM (n = 100).
The series includes 200 index patients diagnosed with HCM and DCM using WHO criteria that accepted to enter our clinical and genetic program on familial cardiomyopathies. The local Ethical Committee has formally approved the project. T-cap gene has been screened by DHPLC and bidirectional sequencing of heteroduplex amplicons.
We identified five heterozygous T-cap gene mutations (5 of 200 patients, 2.5%), four in familial HCM [R106C in two unrelated probands, and 637_640 Del2G in further two unrelated probands] (4%) and one [R63C] in one familial DCM (1%). The mutations were absent in a series of 100 healthy controls.
Telethonin encoding T-cap gene mutations are associated to inherited DCM and HCM: the prevalence is higher in HCM than in DCM. This study provides the genetic epidemiology basis for progressing with further investigations on the role of telethonin in myocardial diseases and definition of precise clinical phenotype associated with these mutations.

P0151. Investigation of “malignant mutations” *MYH7* gene in Hypertrophic Cardiomyopathy in Iranian patients

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Hypertrophic cardiomyopathy (HCM) is characterized by hypertrophy of ventricles and intrventricular septum. Patients could develop serious complications including heart failure, arrhythmias and sudden death. HCM has autosomal dominant inheritance. Methods 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. We find mutation V411I in exon 13, one of them, A10419C (N444T) in exon 14 may be a novel mutation, and also some mutation in intronic region such as IE14 T10630A, IE14 C10663T, E19 R703R, I19 A13573G, I20 C13879G, and I20 C13978A.We are investigating on others exons of this gene.

P0152. Severe hypodontia in siblings

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Background: Isolated severe hypodontia is a rare developmental tooth anomaly and involves the absence of six or more permanent teeth, excluding the third molars. Twin, sibling and familial studies are used to offer more information about genetic and/or environmental determinants of hypodontia. **Objectives**: To analyze the congenital hypodontia pattern in sibs; to find evidence that intrafamilial variation of hypodontia is caused by mutations in the Pax9 gene. **Patients and Methods:** 58 families with hypodontia in two or three successive generations were evaluated clinically and radiographically and three unrelated patients were selected based on their severe phenotype. Their DNA blood samples were analyzed by PCR. Inclusion criteria: patients with severe hypodontia and their siblings who expressed at least one missing permanent tooth; affected biological parents were included too. Exclusion criteria: patients and their siblings with missing teeth due to decay or gum disease. **Results:** the probands and their siblings did not share similar pattern of hypodontia with regard to the tooth class, region, symmetry and number of teeth involved. In all the cases, hypodontia followed a similar pattern of inheritance: autosomal-dominant with incomplete expression and reduce penetrance on the side affected jaw. The association between the presence of a certain mutation and the resulting pattern of hypodontia was not yet confirmed. **Conclusions:** Severe hypodontia phenotype is caused by a large number of different possible mutations.

P0153. Hypohidrotic ectodermal dysplasia in a girl with a 9;X insertion and completely skewed X chromosome inactivation

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X-linked hypohidrotic ectodermal dysplasia (XLHED) is due to a mutation in the *ED1* gene located just proximal to the X inactivation centre. The disorder affects males primarily but carrier females may have mild manifestations. We report a 2-year old girl with severe manifestations of XLHED.

Case report: The presenting complaint was sore eyes and photophobia. Her hair was sparse and thin. She had eyelashes but virtually no eyebrows. Her skin was fair with a tendency to wrinkling under the eyes. She had a single erupted conical maxillary incisor. Three more conical teeth in the same region were evident radiologically. Psychomotor development was normal. Parents were healthy and unrelated.

Laboratory investigations: Routine chromosome analysis was normal. X chromosome inactivation was completely skewed with the paternal X preferentially active. FISH paint X showed an insertion of X material into chromosome 9. Further analyses using BAC probes towards *ED1* and *XIST* confirmed that a fragment of at least 4Mb containing *XIST* was inserted into 9p13 in conjunction with a *de novo* pericentric inversion of chromosome 9. The final karyotype was 46,X X,ins(9;X)(p13;q13q21)inv(9)(p13q13). BAC FISH confirmed that the proximal breakpoint was in the *ED1* gene. The distal breakpoint was distal to the *XIST* locus but was not further mapped. Both parents had normal chromosomes, and the mother had a random X inactivation pattern.

Conclusion: Since *XIST* was lacking on the X chromosome with a disrupted *ED1* gene, the normal X chromosome was inactivated resulting in a severe phenotype of XLHED in this girl.

P0154. Hypomelanosis of Ito and Camptodactyly: A Case Report

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Ibtroduction: Hypomelanosis of Ito (HI) is a disorder characterized by unilateral or bilateral macular hypopigmented whorls, streaks, and patches. Abnormalities of eyes, musculo-skeletal system and of the central nervous system may also occur. Other associated anomalies in hands are clinodactyly, syndactyly, and polydactyly and few cases with HI and camptodactyly have been reported. Case report: 22 months-old female with delayed psychomotor development and short stature. At physical examination her weight wa 6,500 g (-3pc); heigth of 75 cm (-3pc), CFO 47.5 (80-90); thin hair with alopecia in temporal regions, facial dysmorphy characterized by triangular face, wide and prominent forehead, hypertelorism, down-slant9ing palpebral fisures, depressed bridge of the nose, short philtrum, thin lips, low-set and dysmorphic ears; hypopigmented whorls, streaks, and patchy skin lesions that follow the lines of Blaschko on all body. The hands showed fusiform fingers, bilateral camptodactyly in 2-4th fingers, thenar and hypothenar hypoplasia. In the lower extremities there was shortening of the tibia and *hallux valgus* in the right foot. Discussion: The HI (OMIM 300337) is an entity, with a wide clinical spectrum, and the principal differential diagnosis in this case is the Terminal Osseous Dysplasia and Pigmentary Defects Syndrome that is characterized by abnormal and delayed ossification of bones in the hands and feet, brachydactyly, camptodactyly, and clinodactyly, severe limb deformities, joint contractures and pigmentary skin lesions. The others skeletal and skin abnormalities could be explained as part of the variable expressivity in HI. As long as the molecular basis remains unknown, clinical criteria will be the only support for HI diagnosis and classification

P0155. Polymorphisms of Estrogen Receptor Beta Gene Are Associated with Hypospadias

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Introduction: Hypospadias is a common congenital male urethral malformation, defined as the displacement of the urethral meatus ventrally from the tip of the glans penis. The importance of androgen receptor (AR) in male external genitalia development has been well recognized. Recently, the presence of active estrogen receptors (ESR) in the developing male genitalia has been demonstrated. There are two isoforms of the human estrogen receptor, ESR1 and ESR2. The detrimental effects of environmental toxicants with estrogenic activity are mediated, in mice models, by ESR1, leading to feminization of male external genitalia. Besides, it has been shown that individuals with relatively short CA repeat polymorphism in *ESR2* displayed higher androgen levels than those with longer CA repeats. We hypothesized that modifications in these nuclear receptors' genes could lead to hypospadias.

Patients & Methods: We have genotyped the CA repeat polymorphism in *ESR2* and the TA repeat polymorphism in *ESR1* in sixty boys with hypospadias and in a control population.

Results: The CA repeat polymorphism in *ESR2* is prolonged in hypospadias patients than in controls ($P<0,05$).

Discussion: Variations in the *ESR2* seem to influence risk to hypospadias. Longer CA repeat polymorphisms might increas susceptibility to hypospadias due to lower levels of androgens. As ESR2 is likely to be involved in the prenatal sexual differentiation, it is possible that the effects observed are due to early organizational effects of this receptor. Acute effects of these receptors on the hormonal production in the adrenal glands may be an explanation.

P0156. A particular case of syndromatic ichthyosis

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Congenital ichthyoses represent a vast and markedly heterogeneous group of diseases characterized by severe keratinization with intense scaling of the whole integument, often associated with erythema.We present a particular case diagnosed at birth with lamelar erythroderma ichthyosis. Our patient is a 10 years old girl, the tenth child of an unrelated and apparently normal couple, delivered at 36 weeks of gestation, (Wt 2550 gr, Apgar score 6). The patient had a brother and a sister which died in the first days of their life with congenital ichtyosis. Postnatal development was delayed, with short stature and severe mental retardation. The patient evaluation revealed that ichthyosis is associate with craniofacial dysmophism (microphthalmia, anteverted nares, macrostomia, micrognathia, dental caries and enamel abnormalities, ears annomalies), limbs annomalies (arachnodactyly, fingers and toes join contractures, congenital dislocation of hip, bilateral with femoral hypoplasia). The association between ichthyosis, craniofacial dysmorphology, hip dislocation and mental retardation allows us to believe that this case is about a syndromatic ichthyosis. The karyotype was normal and excluded a chromosomal etiology. FISH evaluation for Conradi-Hunermann syndrome and for Kallmann syndrome shown no deletion of the EBP gene on Xp11.23, respectiv the STS gene on Xp22.32. There is the posibility of a dominant ichthyosis caused by a germinal mosaicism of a molecular mutation or a severe form of a recesive ichthyosis caused by a mutation in one of the involved genes. The molecular evaluation can elucidate the case.

P0157. Clinical outcome of 8-year-old ICSI children and spontaneously conceived children

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Background: Although thousands of children are born worldwide after ICSI, there is still a major concern about its safety and the potential risks for the health and future fertility of the offspring.

Methods: The medical outcome of 150 8-year-old children born through ICSI was compared with 147 singletons of the same age born

after spontaneous conception (SC). A full clinical examination was performed with special attention to congenital anomalies and pubertal development. Information about their general health was obtained from the parents by means of a questionnaire.
Results: No significant medical or neurological problem was documented in the two studied groups._Physical examination did not reveal important differences between ICSI and SC children. Weight, heigth, head circumference and Body Mass Index did not differ between the two groups. 15/150 (10%) ICSI children experienced a major congenital malformation compared to 5/147 (3.3%) SC children (RR 2.94; 95% CI 1.09-7.89). Minor malformations were found in 35/145 (24.1%) ICSI children compared to 25/145 (17.2%) in SC children (RR 1.40; 95% CI 0.88-2.21). Pubertal staging was similar in both groups. Genital examination, nearly in all children performed, showed comparable numbers of abnormalities in boys (ICSI 4%, SC 6.6%) and girls (all normal).
Conclusion: Physical examination including a thorough neurological examination revealed reassuring findings. Major congenital malformations appeared to be more frequent in the ICSI group. Overall general health of the children born after ICSI seems satisfactory. Further monitoring of these children regarding further puberty and future fertility is mandatory.

P0158. The Frequency of IL-2, IL-6 and Interferon γ- genes polymorphisms in Hepatitis B patients in Iran

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Background: Cytokines play an important role in the defense against viral infection, both indirectly, through determination of the predominant pattern of the host response, and directly, through inhibition of viral replication. Several pro-inflammatory cytokines such as interleukin-2, interferon-gamma and tumor necrosis factor-alpha have been identified as participating in the viral clearance and the host immune response to HBV. The aim of the present study was to investigate whether the IL-6 (-174), IL-2 (-330), IFN-g (-874) promoter polymorphisms was associated with outcomes of HBV infection in Iranian patients.
Methods: In a case-control study, we examined 96 unrelated patients with Chronic Hepatitis B and 96 spontaneously recovered, were referred to Taleghani hospital. The healthy control group consisted of 96 people who all had been matched by age and sex. The polymorphisms were detected by PCR-RFLP.
Results: The IL-2, IL-6 and IFN-g genotype distribution and allele frequency in patients and controls are shown in Table 1.
Our results show that there is no association between each polymorphisms and chronic hepatitis B patients over spontaneously recovered (p>0.05).
Conclusion: These findings are in contrast with other studied population (Black, Hispanic and Asian), therefore distribution of these polymorphisms are strongly ethnic dependent. Determination of these genotypes is probably not a suitable genetic marker for the risk assessment of HBV in our subject.
Table 1- Distribution of polymorphisms and alleles frequency

polymorphisms		Healthy Controls	Spontaneous recovered HBV	Chronic HB
IL-2(-330)	*T/T* *G/T or G/G* ***Allele Frequency***	27.4% 72.6% ***G=47.8%***	27.1% 72.9% ***G=47.3%***	24% 76% ***G=49%***
IL-6 (-174)	*G/G* *C/G or C/C* ***Allele Frequency***	47.4% 52.6% ***C=28.2%***	58.2% 41.8% ***C=26.5%***	53.6% 46.4% ***C=27%***
IFN-g(-874)	*T/T* *A/T or A/A* ***Allele Frequency***	***26.1%*** ***73.9%*** ***A=60%***	***35.3%*** ***64.7%*** ***A=43%***	***14.5%*** ***85.5%*** ***A=52%***

P0159. The interleukin-10 -627C/A promoter polymorphism is associated with essential hypertension in Tatars from Russia

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Aims. Interleukin-10 is an immunoregulatory cytokine with various actions. Experiments on knockout and transgenic mice showed that it had possible atheroprotective effect. Essential hypertension is thought to be a multifactorial disorder involved in endothelial dysfunction and atherosclerosis. Previously it was reported that *-627 C/A* promoter polymorphism was associated with decreased interleukin-10 plasma level. The aim of the present study was to evaluate the association between the interleukin-10 gene *-627 C/A* promoter polymorphism and genetic susceptibility to essential hypertension.
Materials and methods: We carried out genotyping of 274 men with essential hypertension and 99 healthy subjects from Tatar ethnic group from Bashkortostan, Russia, by polymerase chain reaction restriction fragment length polymorphism method. Statistical analysis was performed using Fisher's exact test, P-value of <0.05 was taken as statistically significant.
Results: The frequencies of *IL-10 -627C/A* genotypes were the following: *CC* 71.72%, *CA* 27.27% and *AA* 1.01% in control group, *CC* 51.81 %, *CA* 39.8% and *AA* 8.39% in hypertensive patients Thus, in Tatar ethnic group *AA* genotype was associated with increased risk of essential hypertension (OR=8.98, P=0,006).
Conclusion: Our results suggest an important role for interleukin-10 in the pathogenesis of cardiovascular disease.

P0160. Two cases with interstitial deletion of chromosome 2p

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Structural deletions of the short arm of the chromosome 2 are rare. We report two cases of interstitial deletion of chromosome 2p.
First patient - physical evaluation at the age of three showed mild dysmorfic feature, low set protruding ears with mongoloid palpebral fissures. He was treated because of bilateral vesicouretheral reflux. His speech was poorly developed, with other signs of mild psychomotoric retardation. Chromosome analysis of blood cells with G-banding showed an interstitial deletion of chromosome 2p. Parental karyotypes were normal. Patients karyotype was 46, xy, del (2) (p12p10) de novo.
Second patient, the two years old boy with several dismorphic features: prominent forehead, mongoloid palpebral fissures, epicanthic folds, broad nasal bridge, short, well formed philtrum, low set protruding ears and bilateral simian crease. He showed signs of mild psychomotoric developmental delay with poorly developed speech. Conventional banding cytogenetics showed an interstitial deletion of chromosome 2p. Karyotyping of the parents revealed that the father carried small ring shaped supernumerary marker chromosome, in addition to the interstitial deletion 2p. The karyotypes of the child's grandparents were normal. FISH identified the marker which consisted of the proximal region of the p-arm of chromosome 2 including a part of its centromere. The final karyotype was described as 47,xy,del(2)(p11.2p10),+ mar de novo. ish der (2)(wcp 2+, D2Z1 +) (ISCN 1995). The rare example shows that our marker chromosome is a part of a balanced karyotype. The father in our case may produce unbalanced offspring and prenatal diagnosis must be recommended.

P0161. Prevalence of *AHI1* and *NPHP1* mutations and genotype-phenotype correlations in a cohort of 25 Dutch patients with Joubert syndrome.

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Joubert syndrome (JBS) is an mental retardation syndrome with hypotonia, ataxia, vermis hypoplasia, oculomotor abnormalities, and a characteristic breathing pattern. Associated anomalies include renal and retinal disease. JBS shows autosomal recessive inheritance,

and is genetically heterogeneous. Mutations in the AHI1-gene or homozygous deletions of the NPHP1-gene have been found in a subset of patients, and linkage to loci on chromosome 9 and 11 has been described.
In a cohort of 25 Dutch patients with JBS we performed DNA-analysis of *AHI1*, *NPHP1,* and the candidate gene *CCND1*. Sequence analysis of *AHI1* revealed DNA-alterations in seven patients. Three patients were compound heterozygous for truncating mutations. All but one were novel mutations not reported before. Two patients were homozygous for two presumably pathogenic missense mutations. In two patients one possibly pathogenic missense mutation was identified.
Homozygous deletions of *NPHP1* were not detected in any of the patients. Sequence-analysis of *CCND1* revealed no mutations.
AHI1 mutations account for 20% of JBS patients in our study population. The five patients that were homozygous or compound heterozygous for *AHI1* mutations all had classical neurological features of JBS and retinal dystrophy. Renal disease or other JBS associated features were not present. The two patients with a heterozygous missense mutation both had retinal colobomas and renal disease. Colobomas have only been described in JBS families that show linkage to chromosome 11. This could mean that the phenotypic spectrum of *AHI1* mutations may be broader than currently assumed.

P0162. Psychiatric and cognitive difficulties - onset of juvenile Huntington disease? Study of 29 patients

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BACKGROUND: Juvenile Huntington's disease (JHD) is a rare clinical entity characterized by an age at onset before 20. Patients usually have an expansion of more than 60 CAG repeats in the HD gene and paternal transmission is the rule. PATIENTS AND METHODS: We retrospectively reviewed clinical and genetic data of JHD patients (n=29) seen at the Referral center for HD of the Salpêtrière hospital, Paris, France, between 1990 and 2005. RESULTS: The mean delay before diagnosis was 9±6 years (0-21). The most remarkable signs at onset were severe psychiatric and cognitive disturbances (19/29, 65.5%), whereas rigidity was absent. Unusual signs at onset included myoclonic head tremor in 3 patients, severe isolated drug or alcohol addiction in 2, psychotic disorder in 1 and difficulty writing in 1. During the course of the disease, psychiatric disturbances were severe with at least one suicide attempt in 7/29 patients. Transmission was maternal in 25%. Forty-six percent of JHD patients had less than 60 CAG repeats, six of whom inherited the disease from their father. Anticipation (18±9 versus 25±11, p=0.27) and age at onset (17.14±2.2 versus 13.29±5.5 years, p=0.086) were similar in patients with maternal compared to paternal transmission. CONCLUSION: Most JHD patients started the disease with psychiatric and cognitive difficulties. This lead to misdiagnosis or diagnosis delay, especially in cases without familial history of HD. Maternal transmissions and expansions of less than 60 CAG repeats were unexpectedly frequent and should not be considered exceptional.

P0163. Kabuki make - up syndrome - clinical study of seven cases

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Kabuki make-up syndrome is a rare disorder characterized by distinctive facial features, dermatoglyphic abnormalities, short stature and mental retardation. Some cases associate cleft palate, cardiovascular and genitourinary defects and recurrent infections. Most cases are sporadic. The underlying genetic mechanism remains unknown.
We have analyzed the clinical picture and investigations in 7 children diagnosed with Kabuki syndrome (two girls and five boys). All the cases associated ocular and auricular defects (long palpebral fissures, eversion of lower lateral eyelid, arched eyebrows, long eyelashes and large/ prominent ears, fetal pads and mental retardation. Postnatal growth retardation with microcephaly, micrognathia and heart defects (ASD, VSD, dextroposition) were recorded in 71,74% of the cases. Half of the patients associated also short nose and increased susceptibility to infections. Rare defects recorded in our cases were: cleft/ high-arched palate, dental abnormalities, brachydactyly, irregular toes implantation, joint hyperextensibility and genitourinary abnormalities. Some particularities (e.g. heart dextroposition, shawl scrotum and renal failure) will be presented. The severity of the disorder will be analyzed according to the presence/ absence of some factors. A comprehensive differential diagnosis will complete the presentation.
In conclusion we did a clinical study of 7 cases with Kabuki make-up syndrome and found in all of them ocular and auricular abnormalities, fetal pads and mental retardation. Other frequent features are postnatal growth retardation with microcephaly, micrognathia, heart defects and increased susceptibility to infections. Patient's prognosis depends on the presence/ absence of cardiac defects, renal failure, and severity of mental retardation.

P0164. Kallmann syndrome can be caused by mutations in *CHD7*

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The combination of hypogonadotropic hypogonadism with anosmia due to olfactory bulb a/dysplasia, is known as Kallmann syndrome (KS). Associated features can be bimanual synkinesia, renal agenesis, cleft lip/palate, dental agenesis and hearing loss. Two genes, responsible for KS in approximately 20% of all cases, have been identified: *KAL1*, involved in the X-linked form (KAL1, OMIM #308700) and *FGFR1,* causing the autosomal dominant form (KAL2, OMIM #147950).
CHARGE syndrome (OMIM #214800) is characterized by a variety of congenital anomalies, including renal agenesis, cleft lip/palate and hearing loss. *CHD7* on chromosome 8q12.1 was discovered as the major gene involved in CHARGE syndrome. Recent studies showed that anosmia and hypogonadotropic hypogonadism are consistent findings in CHARGE syndrome as well. Therefore, we investigated whether mutations in *CHD7* can also cause KS.
Patients with a clinical diagnosis of KS, without mutations in *KAL1* or *FGFR1* were screened for *CHD7* mutations. None of the patients fulfilled the diagnostic criteria for CHARGE syndrome. Sequencing of *CHD7* has been performed in six patients so far. This revealed a nonsense mutation in one KS patient with perceptive deafness and hypodontia, and a missense mutation in two KS patients without associated features. These mutations have not been described in CHARGE syndrome previously, nor have they been marked as polymorphisms. These preliminary data (more patients are to be sequenced and testing of the parents will reveal whether these mutations are *de novo*) suggest that the spectrum associated with *CHD7* mutations includes (variant) Kallman syndrome.

P0165. A familial case of Kartagener's syndrome revealed by azoospermia in an infertile man

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Primary ciliary dyskinesia is a rare etiology of infertility in man. It is an autosomal recessive disease characterized by defective cilial ultrastructure in ciliated cells and affects about 1 in 20000 newborns. Regarded as a subgroup of primary ciliary dyskinesia, Kartagener's syndrome is characterized by the simultaneous presence of chronic bronchorrhea with bronchiectasis, chronic sinusitis and situs inversus. Infertility, occasionally described in males, is caused by ultrastructural defects of sperm tails with always total asthenozoospermia.
We report a consanguineous family with two siblings, a male and a female, who have a typical Kartagener's syndrome and another dead brother who seems to be affected. The diagnosis was established on the basis of clinical grounds and familial history of a 33-year old man who was referred for cytogenetic exploration because of 11 months history of male infertility with azoospermia in semen analysis. Clinical history and physical examination revealed presence of situs inversus totalis, chronic bronchorrhea and bronchiectasis, chronic nasal

symptoms, sinusitis and infertility. Serum FSH, LH and testosterone were normal and the patient had a normal 46,XY karyotype. The patient's sister was also affected and had similar features with the same respiratory symptoms and situs inversus totalis. After some difficulties to conceive, she gave birth to a healthy female child and a male stillborn with multiple congenital abnormalities. Their third sister had also history of foetal congenital abnormalities. The infertile patient who sought an ICSI treatment underwent genetic counselling in which he was informed about all possible risks.

P0166. Recurrent and novel mutations in human matrix Gla protein in Keutel syndrome.

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Keutel syndrome (KS; MIM 245150) is an autosomal recessive disorder with abnormal calcification of cartilage (airways and ears), short distal phalanges, and facultative peripheral pulmonic stenosis. Four different mutations in MGP, the gene coding for Matrix-associated GLA protein, have been identified to date.

We have sequenced the MGP gene in 7 individuals with tracheobronchial cartilage calcifications and brachytelephalangy, and found mutations in 4 of them. Two girls of Turkish origin were homozygous for IVS1-2A>G, previously observed in another Turkish family. One male patient, also born from Turkish parents, was homozygous for a novel IVS1+1G>A mutation, located in the donor splice site at the exon1-intron1 junction. The fourth patient, a female of Arab origin, was homozygous for Y29X, previously found in a Belgian family. Unlike MGP-negative patients, all families with MGP mutations were consanguineous. All patients had normal stature and none had neurological symptoms.

We conclude that: 1) the association between MGP mutations and the KS phenotype is confirmed; 2) all MGP mutations predict a non functional protein, consistent with the known dose-dependent modulation of bone morphogenetic protein by MGP; 3) unlike most other disorders with excessive calcification, notably chondrodysplasia punctata in its variants, short stature is not a feature of KS; 4) the paucity of reported cases, the frequency of parental consanguinity, and the presence of a recurrent mutation in Turkish families all seem to indicate that KS is significantly rarer than other disorders with abnormal cartilage calcification, although it may be comparatively less rare in Turkey.

P0167. Klinefelter syndrome associated with moderate mental retardation, seizures and isolated growth hormone deficiency - a case report

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Klinefelter syndrome (KS) is the most common cause of hypogonadism. The boys usually have borderline intelligence, behavioral problems, tendency towards tall and disproportionate habitus. Associations between KS and other conditions are described.

A boy of 12 years has been referred to our clinic due to statural deficiency, hypogonadism and small testes. From the age of two years he had seizures refractory to several combinations of anticonvulsant drugs. Dysmorphic features on his face included high forehead, deep-set eyes, broad and long nose, pointed chin. His height was 3 SDS below the mean, long arms and legs in accordance to the body height. His IQ was 40 and he had speech impairment and behavioral problems such as insecurity and shyness. Karyotype was 47, XXY. Because of short stature, hormonal tests for growth hormone have been performed; all with the values below 2 ng/ml. MRI of the brain was normal. Hormonal replacement therapy was not performed because of the risk for refractory seizures. However, follow up of the child showed satisfactory growth velocity and spontaneous appearance of pubertal signs. Favorable outcome of the height without a hormonal replacement therapy is due to the tendency towards higher growth in persons with KS.

According to our knowledge, this case represents a rare association between Klinefelter syndrome, growth hormone deficiency, and refractory seizures. Since additional abnormalities of the brain, especially hypophyseal region were not discovered, the reason for this association remains undiscovered. Concomitant presence of other condition associated with seizures and IGHD with KS is possible.

P0168. Klinefelter Syndrome associated with a prolactin-secreting adenoma

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Klinefelter syndrome is the most common form of male hypogonadism. It is a genetically determined primary gonadal defect characterized by the XXY karyotype. Pituitary changes in patients with Klinefelter syndrome have not been evaluated in detail. In fact, protracted stimulation of gonadotrophs due to lack of androgen feedback might have been a factor in the formation of the gonadotroph adenoma or in the development of gonadotroph hyperplasia. Clinically silent GH microadenoma was also described. At our knowledge, only one case of association between Klinefelter and prolactin (PRL)-secreting adenoma was reported. We report here a new case of this association.

A 16-year-old patient who consulted for gynaecomastia. Laboratory findings showed hypogonadism with high concentrations of FSH and LH (FSH=96 mUI/l and LH=38 mUI/l) and the diagnosis of Klinefelter syndrome was confirmed by cytogenetic testing. However, the level of serum prolactin was high (PRL= 1030µUI/l) and the MR imaging pituitary showed a non-invasive macroadenoma with enlarged sella turcica

In conclusion, clinical features in Klinefelter syndrome are commonly caused by peripheral hypogonadism. However, an association with hyperprolactinemia must be researched.

P0169. Kyphomelic dysplasia in a female infant with a de novo terminal Xp22.33-p22.22 deletion and skewed X-inactivation

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We report on a female infant with kyphomelic dysplasia and a de novo deletion of the short arm of the paternal chromosome X. The patient had skewed inactivation of the X chromosome carrying the deletion. None of the clinical features of the syndromes located in the deleted region were present. The systemic skeletal disorder showed rhysomelic and mezomelic limb shortening, flaired ribs and skin dimples over the bony prominences. Although the child had muscle hypotonia, no episodes of apnoe, feeding difficulties or temperature instability were observed. Kyphomelic dysplasia is genetically heterogeneous, and has been previously suggested to have an autosomal recessive inheritance. According to earlier reports 14 out of 19 reported cases with the typical radiological finding were males. Our findings support a possible X-linked inheritance of this rare condition.

P0170. Clinical spectrum of late-onset cobalamin C disease including three new cases and follow-up of two previously described cases with a review of the literature

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Cobalamin C disease (Cbl-C) is the most common of the inborn errors of cobalamin metabolism with recessive autosomal mode of inheritance and *MMACHC* gene mutations. Clinical features occur usually in the first year of life including failure to thrive, microcephaly, poor feeding, vomiting, hypotonia, mild to moderate developmental delay, speech delay, and seizures. Some patients also present with macrocytic or microcytic anemia, hypersegmented neutrophils, thrombocytopenia, and microthrombotic disease. Besides to the neonatal form, an adult onset have been described with 10 cases reported in the literature to date. Here, we report on three new late-onset cases, the follow-up of two previously reported cases and reviewed the literature. Clinical features include predominant neurological disturbances (12/13 cases) with myelopathy, encephalopathy and psychiatric disturbances, thromboembolic disease (8/13 cases), visual complications (9/10 cases) including optic pallor and renal involvement (5/13 cases). Neuroradiological investigations were not always contributive (6/11 cases), white matter abnormalities being the more prevalent features (4/6 cases). None of the patients had abnormal hematological signs and serum vitamin B12 levels. The hydroxycobalamin therapy frequently led to a neurological improvement (11/13 cases). Four patients died of vascular complications, two being treated and two untreated. Because of easy diagnosis by amino acid chromatographies and favorable neurological and vascular outcome with simple therapy by hydroxycobalamin, Cbl-C deficiency should be included in the differential diagnosis of patients with progressive neurological deterioration, psychiatric disturbances and recurrent thromboembolic complications.

P0171. Triallelism in Leber Congenital Amaurosis: a clinical case

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INTRODUCTION:Leber Congenital Amaurosis (LCA) is the most severe inherited retinopathy with the earliest age of onset. Non syndromic LCA has been associated with mutations in eight genes: AIPL1, CRB1, CRX, GUCY2D, MERTK, LRAT, RPE65 and RPGRIP1. These genes are involved in different physiologic pathways in the retina.

MATERIAL AND METHODS:We report a mutational analysis of a Retinal Dystrophy family with two affected generations (four affected members) with differnt phenotypic severity: one LCA affected patient in the youngest generation and three early onset RP affected patients en two sibship of the oldest generation. They were studied with a genotyping microarray (disease chip) for LCA (www.asperbio.com) and have been further refined with the use of additional polymorphic markers (STRs) and direct sequencing.

RESULTS:The patient affected with LCA presents three mutated alleles; two mutations in CRB1 gene (C896X, found by LCA chip and E1330N, newly found by direct sequencing) and the third one in RPGRIP1 gene (Q589H, found with LCA microarray). The other affected members of the family present E1330N (CRB1) mutation as well as the RPGRIP1 mutation.

Haplotype analyses are compatible with CRB1 segregation in this family and with the existence of a second CRB1 mutated allele in the three affected RP patients.

CONCLUSION:In this family the presence of two CRB1 mutated alleles and RPGRIP1 mutation in every case is associated with a severe but variable RP phenotyope.

We propose that the RPGRIP1 mutation could affect the course of the disease but it does not explain the variability in this family.

P0172. Facial dysmorphism in Leigh syndrome with SURF-1 mutation and Cytochrome C Oxidase deficiency

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Leigh Syndrome is an inherited, progressive neurodegenerative disorder of infancy and childhood. Mutations in the nuclear SURF-1 gene are specifically associated with cytochrome c oxidase deficient Leigh syndrome

Here, we describe two patients with similar facial features. One of them was 2 and a half year-old boy and the other was three year- old boy with a mutation in SURF-1 gene and facial dysmorphism included frontal bossing, brachycephaly, hypertrichosis, lateral displacement of inner cani, esotrophia, maxillar hypoplasia, hypertrophic gums, irregularly placed teeth, upturned nostril, low set big ears and retrognathi. First patient at 15 month-old his first MRI showed mild symmetric T2 prolongation involving the subthalamic nuclei.His second MRI at 2 years old revealed a symmetric T2 prolongation involving the subthalamic nuclei, substansia nigra and medulla lesions. In second child at the age of 2; his first MRI showed heavy brain stem and subthalamic nuclei involvement. A second MRI was performed when he was 3 years old and showed diffuse involvement of substansia nigra and hyperintense lesions of central tegmental tract in addition to previous lesions. Facial dysmorphism and MRI findings noticed in these cases, can be specific findings in Leigh Syndrome patients with cytochrome c oxidase deficiency. SURF-1 gene mutations must be particularly reviewed in such patients.

P0173. Molecular evidence against a role of the *NF1* gene mutations in LEOPARD syndrome

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LEOPARD syndrome (LS) is a rare autosomal dominant condition, mainly characterised by facial dysmorphisms, congenital heart defect, in particular hypertrophic cardiomyopathy, multiple lentigines and cafè-au-lait spots (CLS). Up to 90% of LS patients present missense mutation in *PTPN11* gene, encoding for the SRC homology 2 (SH2) domain-containing PTPase (SHP-2). Several clinical manifestations overlap those of Noonan syndrome (NS), which is due to different *PTPN11* mutations. In paediatric LS patients, CLS and/or lentigines pose difficult differential diagnosis mostly with Neurofibromatosis type 1 (NF1) and NF/NS, largely caused by *NF1* gene mutations. To date, no other gene mutation has been concretely related to LS, even if a mutation in *NF1* gene was reported in a patient supposed to be affected by LS. Increasing evidence suggests that SHP-2 and neurofibromin, the *NF1* gene product, play their modulatory role through a common pathway. This evidence prompted us to screen the *NF1* gene in 4 full-blown LS individuals, in which *PTPN11* gene mutations had been excluded by DHPLC and sequence analysis of the entire coding region. The *NF1* gene analysis was carried out by DHPLC and sequencing. No pathogenic mutation was detected in our LS individuals. This result indicates that *NF1* gene mutations are not related to LS, thus implying that the clinical overlap between LS and NF1-NFNS is not paralleled by a unique molecular event.

P0174. Linkage Analysis of some of the DFNB loci in Non-Syndromic Autosomal Recessive Hearing Loss in two provinces of Iran

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Congenital deafness is a frequent disorder that affects 1 in 1000 neonates with about 50% hereditary form. Almost 60 autosomal recessive hearing loss loci has been reported sofar. The main idea of this study is to find the contribution of some of the DFNB loci and the connexin-26 gene mutations (exon2) on the Iranian hearing loss in the Markazi and Qom provinces.

Forty families, included nearly 450 samples, with autosomal

recessive congenital hearing loss with at least 3 affected individuals and consanguineous marriage were selected. DNA samples were amplified by using the specific primers for coding region of connexin-26 gene (exon2) and the specific primers for at least 2 STR markers for each locus that were exceeded to 4 markers in the linked families. The PCR products of the connexin-26 gene were quality controlled on the Agarose gel and sequenced. The PCR products of the STR markers were analysed by Polyacrylamide Gel Electrophoresis.Six families were homozygous or compound heterozygous for the Connexin-26 gene coding region and were excluded from linkage analysis. The 34 remained families were genotyped for STR markers from DFNB2, DFNB3, DFNB4 and DFNB21 loci to find the role of these loci. We found linkage in 5 families: One to DFNB2, two families to DFNB3 and two others to DFNB4 loci, while none of the studied families showed linkage to DFNB21 locus. Hopefully some novel mutations will be found in the linked families by direct sequencing the important genes for deafness into those regions.

P0175. Sixteen years experience of biochemical analysis and prenatal diagnosis of Lipid storage disease in Iran

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Lipid storage diseases are a group of inherited metabolic disorders in which harmful amounts of fatty materials (called lipids) accumulate in some of the body's cells and tissues. Over time, this excessive storage of fats can cause permanent cellular and tissue damage, particularly in the brain, peripheral nervous system, liver, spleen, and bone marrow. Lipid storage diseases are inherited from one or both parents who carry a defective gene. Neurological complications of the lipid storage diseases may include ataxia, eye paralysis, brain degeneration, seizures, learning problems, spasticity, feeding and swallowing difficulties, slurred speech, loss of muscle tone, hypersensitivity to touch, burning pain in the arms and legs, and clouding of the cornea.
As an oldest reference laboratory, from Aug. 1989 to Aug. 2005 we study 82 families with 114 members affected with LSD. Lipid storage diseases which were analyzed were Metachromatic leukodystrophy, Niemann-Pick disease, Gaucher disease, Mucolipidisis, Canavan, Alexander, GM1-Gangliosidoses, GM2- Gangliosidoses (include Tay-Sachs disease and Sandhoff), Fabry disease, Krabbe disease, Fucosidosis, Wolman's disease, Neuronal ceroid lipofuscinosis, Sea blue histiocytosis, Lipidosis. We performed a total of 36 prenatal diagnoses from 77 couples at risk for LSD. Biochemical assays were applied for all cases. Twenty-three fetuses (%64) were found to be normal, 12 fetuses (%33.4) were affected and result of one of the samples is not ready yet. Our data supports the functionality of biochemical analysis and for these diseases of course when the families with affected alive child referred to the diagnostic centers before loose of their affected child.

P0176. Diagnostic Criteria for Congenital Long QT Syndrome (LQTS); Re-appraisal of the Schwartz' Criteria

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Introduction: LQTS is a primary inherited cardiac arrhythmia syndrome which may cause sudden death in young individuals. Because the clinical diagnosis is not always obvious, a set of diagnostic criteria was formulated in 1993, the "Schwartz criteria" (SC).
Purpose : Evaluation of the predictive value of the SC in relatives of probands who carry a LQTS gene mutation, using molecular genetic testing as gold standard.
Methods and Results: Since 1996 we identified 96 probands with a disease causing mutation. 517 genotyped relatives were included in this study.
In relatives with a positive genetic test 40/212 had a SC ≥ 4 (sensitivity 19%, specificity 99%). Measuring QTc duration alone in 517 relatives revealed a QTc duration ≥ 430 ms as the optimal cut-off point to predict the genotype, with an area under the curve of 0,848. Sensitivity and specificity measured at QTc duration ≥ 430 ms are 71% and 86% respectively. The rate of false positives increases when heart rate exceeds 75/min or CL < 800 ms.
Conclusions: The Schwartz score has low sensitivity in identifying carriers of an aberrant gene in families with an established disease-causing mutation. Analysis of QTc duration alone (QTc ≥ 430 ms) has a sensitivity of 71% and a specificity of 86% in predicting mutation carriership (AUC 0.848). An important cause of false positive results using this method is a relatively fast heart rate; the specificity of this method increases to 93% when relatives with a heart rate above 75 bpm (CL < 800 ms) are excluded.

P0177. The natural history of the Long QT syndrome type 1 and Long QT syndrome type 3: family tree mortality ratio method.

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PURPOSE: To estimate all cause mortality ('natural history') with the established Family Tree Mortality Ratio Method in untreated patients with inherited arrhythmia syndromes in times when the disease, therapy nor the genetic basis was yet recognised, in order to assess whether screening strategies and prophylactic treatment are needed in these patients.
METHOD: One large pedigree with carriers of the G189R mutation in the KCNQ1 gene was obtained (55 persons). Another large pedigree with carriers of the 1795insD mutation in the SCN5A gene was obtained (179 persons). All persons in the pedigree had a 100 % or 50% probability of carrying the mutation. All cause mortality was compared to the general Dutch population in similar time intervals.
MAIN OUTCOME MEASURE: Standardized Mortality Ratio (SMR), the ratio between observed and expected mortality.
RESULTS: For LQTS1 patients; there was significant excess mortality in males (SMR 1.9,CI 1.2-2.9), especially in the age categories 0-1 (SMR 3.5,CI 1.3-7.6) and 1-10 years old (SMR 3.4,CI 1.3-7.4).
For LQTS3 patients; there was significant excess mortality in the pedigree (SMR 1.4, CI 1.03-1.87), especially in the age categories 10-20 (SMR 3.8, CI 1.1-8.2), 20-30 (SMR 6.1, CI 2.9-11.2) and 30-50 (SMR 2.7, CI 1.2-5.3) years old.
CONCLUSIONS: In this study on the natural course of two types of Long QT syndrome, significant excess mortality was observed in different age categories. The observed age distributions are consistent with the literature. Based on these results, continuation of active cascade screening, starting at young age, in LQTS families is justified.

P0178. Genotype-phenotype effect in patients with *LRP5* mutations

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Mutations in *LRP5*, coding for the low density lipoprotein receptor-related protein 5, have been shown to cause a variety of skeletal disorders including autosomal recessive Osteoporosis-pseudoglioma syndrome (OPPG) and autosomal dominant High Bone Mass (HBM) disorder. While homozygous *LRP5* mutations result in OPPG, characterized by severe osteoporosis and blindness, heterozygous *LRP5* mutations may result in milder osteoporosis. In this study we have assessed skeletal phenotypes and *LRP5* mutations in a large Finnish OPPG-family.
DNA samples were collected from 37 individuals, two of whom had OPPG. The exons and exon-intron boundaries of *LRP5* were sequenced. Skeletal phenotype was assessed by fracture history, bone mineral density (BMD) and spinal radiographs. Two different missense mutations were identified. R570W was found in seven individuals and R1036Q in four individuals. The two patients with OPPG were homozygous for R570W, one individual was a compound heterozygote for both mutations, four were heterozygous for R570W and three heterozygous for R1036Q.
The two patients homozygous for R570W were blind and had severe osteoporosis and multiple compression fractures. The compound

heterozygote individual had severe osteoporosis but normal vision. All heterozygous carriers had normal vision but their BMD was notably decreased and several had spinal compression fractures.
In conclusion, the skeletal and ocular phenotypes vary depending on the type and combination of *LRP5* mutations. Even heterozygous mutation carriers may develop severe skeletal complications and should be assessed for osteoporosis.

P0179. Macrocephaly-Cutis Marmorata Telangiectatica Congenita: A Review of 14 Patients With Attention to Clinical Features and Management

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Macrocephaly-Cutis Marmorata Telangiectatica Congenita (M-CMTC) is a rare overgrowth syndrome with a distinctive pattern of clinical findings. In recent years, the understanding of medical concerns associated with this syndrome has expanded. We present clinical findings from 14 unpublished patients with M-CMTC and compare them to cases already in the literature. Our series illustrates the pattern of characteristic findings while underscoring the unique issues in individual patients. Seven of our patients had a Chiari I malformation, and four underwent surgical foramenectomy for concern of symptomatic brain stem compression. In some, cerebellar tonsillar ectopia recurred despite surgical intervention, possibly due to ongoing brain overgrowth. These findings support the recent observations made by Garavelli et al (2005) that Chiari I was previously underappreciated in this syndrome. It is widely suspected that M-CMTC carries a tumor risk for affected patients but the incidence and type of tumor predisposition is not well understood, thereby complicating anticipatory care recommendations. One of our patients developed a cerebral mass with radiographic appearance of a meningioma at age five years. Another unexpectedly developed multifocal atrial tachycardia, a rare arrhythmia, with secondary cardiomyopathy. Similar cardiac complications were previously reported by Yano and Watanabe (2001). Based on literature reports, M-CMTC apparently carries a risk of sudden demise due to a variety of causes. Cardiac arrhythmia, neurologic sequelae, and respiratory failure are known concerns. Determining which patients are at risk is difficult. We propose care management guidelines for this syndrome based on available clinical data and observed complications in reported patients.

P0180. Congenital anomalies and dysmorphic features in patients with oxydative phosphorylation disorders

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Congenital anomalies and dysmorphyc features are seldom reported in oxidative pshophorylation disorders. We performed a prospective study in 30 children diagnosed with a dysfunction of the oxidative phosphorylation and a known mitochondrial or nuclear DNA mutation (22 and 8 children, respectively) to assess the occurrence of associated malformations and minor anomalies. All patients underwent a standard organ screening protocol already in the primary diagnostic phase prior to the muscle biopsy. A skeletal survey was performed additionally in five cases. After the elucidation of the underlying genetic etiology, the same physician reevaluated the children. Congenital malformations were detected in 2 males; one had a skeletal dysplasia, the other had a pulmonary stenosis. Dysmorphic facial features were observed in three females with a *SURF1* mutation presenting with Leigh syndrome, one male with Barth syndrome (*TAZ* mutation), one male with *POLG* mutation and Alpers syndrome and one male with *ND7* gene mutation (mitochondrial myopathy). Apparently dysmorphic features were more common in association with nuclear DNA mutations. Hypertrichosis was present in a high percentage of the cases, especially in those with Leigh syndrome (*ATP6* and *SURF1* mutation). In both patients diagnosed with congenital malformations a second mutation was discovered (*LIFR* and *ELN*) giving explanation for the complex clinical picture.

P0181. Maternal uniparental heterodisomy of chromosome 14 in a young woman with psychiatric manifestations.

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Uniparental disomy (UPD) is defined as the presence of both homologs of a chromosome pair inherited exclusively from one parent. UPD of chromosomes containing imprinting regions leads to clinically recognizable syndromes and maternal UPD of chromosome 14 is one that causes a distinct disorder. It presents with congenital hypotonia, pre and postnatal growth retardation, precocious puberty, scoliosis... No psychiatric manifestations have been reported so far in this disease.
Here, we report the case of a young woman with psychiatric manifestations. Her personal history and physical examination revealed a maternal uniparental disomy of chromosome 14 confirmed by molecular biology despite a normal karyotype.Psychiatric manifestations have never been described in any cases of maternal UPD of chromosome 14 but most cases in the literature concerned young children and such episodes may likely appear later. It is not known if this clinical finding is related or coincidental to the uniparental disomy but we encourage clinicians to pay attention to behavioural disorders or psychiatric manifestations during clinical follow up of maternal UPD of chromosome 14 patients to precise whether they are part or not of this syndrome.

P0182. Maternal uniparental disomy of chromosome [mUPD(14)] in a boy with rob(13;14)mat.

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mUPD(14) is associated with a distinct phenotype, although by no means pathognomonic. A clinical overlap is seen with Prader-Willi syndrome.
The most characteristic features of mUPD(14) are IUGR, hypotonia, short stature, short hands and feet, early puberty, and motor developmental delay. Early puberty is the most consistent feature of this syndrome appearing in 24 of 25 cases reported to date.
A 11 year old boy was referred to our clinic because of developmental delay. He was the third son of young, healthy, unrelated parents.
Clinical evaluation: proportionate short stature, postnatal microcephaly, short hands and feet, early puberty and hypertrichosis.
GTG banding karyotype revealed a rob(13;14); one brother, mother, two maternal uncles and maternal grandmother were also carriers of this translocation.
DNA study with 14q-specific polymorphic markers demonstrated absence of paternal contribution for the regions studied, thereby establishing the diagnosis of mUPD(14).
For most markers a pattern consistent with maternal heterodisomy was observed, whereas for the remaining markers the homozygous pattern observed in the patient indicated isodisomy. This pattern (coexistence of segmental isodisomy and segmental heterodisomy) can be explained by meiotic crossing-over.
In this case the clinical pattern observed is consistent with previously reported cases of mUPD(14).
This case illustrates the importance of checking for UPD whenever a robertsonian translocation involving a chromosome with known imprinted genes (e.g. 14 or 15) is found in prenatal diagnosis or postnatally in a patient with phenotypic alterations.

P0183. The prevalence of the MEFV gene mutations in Armenians

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The main aim of clinical and laboratory investigations relates to the registration and distribution of structure and frequency of genetic pathology in Armenian population. Genetic testing is used for many genetic disorders, including birth defects, autoinflammatory, neurological disorders, cancer, etc. Spectrum of diseases detected by DNA techniques is specified in aspect of more importance and actuality.

We carry out the complete clinical and molecular genetic analysis of the pathogenesis in advanced to understanding inflammation processes and its regulation. Familial Mediterranean fever (FMF), is a recessively transmitted disorder restricted to Mediterranean populations. 12 MEFV gene mutations are identified in 6000 Armenian patients. We have determined symptoms associated with MEFV mutations in 317 heterozygotes, compared with affected 414 homozygotes and 980 compound heterozygotes. Results of screening of MEFV mutations in FMF patients in comparison with healthy individuals have revealed the most frequent mutations and genotypes, and give the information of mutation carriers and genotype - phenotype correlation. These correlations are of great importance since they take into account each clinically significant symptom and different combination of them, which cover almost all of the reported cases. In heterozygote patients the most prevalent and severe cases are caused by the presence of a single M694V mutation, which is associated with main symptoms. We suggest that MEFV heterozygotes suffer from a milder FMF.

We found that on average, in almost 90% of the cases, Colchicine is effective to keep the inflammation under control.

P0184. Megalencephalic leucoencephalopathy with subcortical cysts: phenotype of two patients with new MLC1 mutations.

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Megalencephalic leucoencephalopathy with subcortical cysts is an autosomal recessive leukodystrophy characterized by progressive macrocephaly and slowly progressive deterioration of motor functions. Cognitive decline is moderate and late. MLC1 gene is the major gene of MLC. More than 40 different mutations were reported without any hot-spot. Here we described two non related patients with MLC1 mutations (1 male, age 2 $^{4/12}$ years; 1 female 2 $^{9/12}$ years). Both were born from consanguineous parents. OFC was normal at birth (36 cm) with progressive macrocephaly in the fist year (+5sd, +4sd). In one patient first symptom was non febrile convulsion at age 1 year but diagnosis was suspected only at 2 years when a status epilepticus occurred. Walking was achieved at 18 months and was ataxic. In the second patient disease was revealed by coma and motor regression after head traumatism in association with leucodystrophy on CT-scan. Retrospectively psychomotor delay was present before the traumatism. Recovery of motor functions was slow and incomplete. In both patients MRI showed typical diffuse leucodystrophy with temporal cysts. MLC1 sequence analysis revealed 2 new homozygous mutations.

P0185. Melas in families

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Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis, and Stroke-like episodes (MELAS/OMIM #540000) syndrome is a relatively frequent mitochondrial disorder. It is a multisystemic disorder with an extremely variable clinical phenotype and can be caused by different mutations in the mitochondrial DNA. The classical A3243G mutation in the *MTTL1* (tRNA for leucine (UUR)) gene is most common. We present the clinical features and molecular findings in five families with a mtDNA A3243G mutation. The clinical phenotype in families A, B and C was characterized by proximal muscle weakness, ptosis, external ophthalmoplegia, retinitis pigmentosa, hearing loss and diabetes mellitus. One patient died of heart failure (cardiomyopathy) and one had tachycardia in early adulthood. Family D presented with maternally inherited diabetes mellitus. The first sign in family E was severe hypotonia, developmental delay, muscle weakness, poor growth and elevated lactate in early childhood accompanied by external ophthalmoplegia. Investigation of the A3243G mutation in blood, muscle, hair roots and urine sediment was necessary for diagnosis in patients with nearly no complains. There was no strong correlation between the clinical picture and proportion of mutated mtDNA in these families. Clinical manifestations in MELAS usually become evident when a threshold percentage of mtDNA is mutated, but is not simply a direct consequence of the relative abundance of mutated mtDNA. Other factors such as nuclear background might contribute to the phenotype. Although ethical considerations are being made, preimplantation genetic diagnosis could be an option for few patients with low mutation load in blood and muscle.

P0186. Gene expression changes in skeletal muscle from MELAS patients

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Mitochondrial encephalomyopathies are genetically and clinically heterogeneous disorders. Mutations in different genes can lead to similar syndromes; in contrast, one single mutation can result in a variable clinical manifestation. The most frequent cause (80%) of Mitochondrial Myopathy, Encephalopathy, Lactic Acidosis and Stroke-like episodes (MELAS) is the m.3243A>G point mutation in the mitochondrial $tRNA^{Leu(UUR)}$ gene. Other clinical manifestations of this mutation are diabetes, deafness, renal tubulopathy and cardiomyopathy. To explain the differences in pathology observed in carriers of the m.3243A>G mutation, we applied global gene expression profiling on muscle biopsies from affected and unaffected mutation carriers with different levels of the mutation and controls. Protein turnover and reactive oxygen species (ROS) defence were significantly up-regulated pathways and changes were more prominent in the unaffected group with lower mutation levels than in the affected group. Increased production of ROS between m.3243A>G mutation carriers and controls was further demonstrated by dihydroethidium (DHE) staining of superoxide radicals in muscle. We hypothesise that the accumulation of oxidative protein damage due to excessive ROS production stimulates protein turnover. Apparently, this process is more effective at lower mutation levels where the increased ROS production can be dealt with by increasing the expression of genes involved in protein breakdown and protein synthesis, preventing severe symptoms from occurring. If mutation levels get too high, compensation is no longer possible and severe symptoms become manifest.

P0187. Mental retardation in childhood: etiological and clinical profile

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Mental retardation (MR) is a lifelong disorder of cognitive and adaptive functions. It is present in 2-3% of the general population and results from genetic factors, environmental events or both of them. Aim of the present study was to investigate the etiological factors of MR in children attending a pediatric clinical genetic department and to analyze the clinical signs in each etiologic group. The information of 309 consecutively hospitalized children with MR was analyzed and

overviewed. Etiological diagnosis was established in 144 (46,6%) of the patients. In 78 (25%) a chromosomal disorder was found (40 of them with Down syndrome). Diagnosis of a monogenic disorder was made in 46 patients (14,9%) (34 with monogenic dysmorphic syndromes and 12 with metabolic diseases). Isolated brain anomalies (multifactorial MR) were found in 5 patients (1,6%). Environmental acquired MR was ascertained in 15 patients (4,9%). In 165 patients (53,4%) the diagnosis remained unclear despite the broad spectrum of diagnostic tests carried out (idiopathic MR). Nearly 100% of the diagnosed patients had syndromic MR. The diagnostic yield did not correlate with the severity of MR.

P0188. Risk factors of mental retardation in referral patients to Medical Genetic Counseling Center in Iranian province of Hormozgan (2000-2005)

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This abstract includes a retrograde review of 187 medical files of mental retarded patients that were referred to Medical Genetic Counseling Center in Hormozgan province in south part of Iran that lies in north of Persian Gulf from year 2000-2005.
Results are as fallows:
-From 1523 cases that came to our center for genetic counseling,187 cases (%12.3) had mental retardation in their children.%53 of mothers and %39 of fathers were uneducated.
-%51.8 of parents had consanguinity marriage that among them first cousin marriage were commonest (%70).In the 95 cases (%50.8) the families had more than 3 children.Among mental retarded paitents 107 cases
(%57.2) were male and 80 cases (%42.8) were female.
-In 29 cases (%15.5) maternal age in pregnancy was more than 35 years.
-In 81 cases (%43.3) there was positive family history of mental retardation.
-In 26 cases (%13.9)there was an affected sibling.%46.6 of mothers had prenatal care in pregnancy and %53.4 had no prenatal care.In %57.7 delivery had done in hospital and in %42.3 delivery had done in home without presence of physician.In %27.8 there was a history of neonatal seizure.In %25.5 of cases there was history of birth asphyxia. In%11.7 there was history of neonatal hyperbilirubinemia.
-%3.7 of patients were born preterm.
-The commonest diagnosis among the mental retarded patients was Down syndrome (%17.1).

P0189. Recurrent submicroscopic copy number changes in 2q23.1q23.2 in mentally retarded patients

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Routinely applied microarray-based genomic profiling technologies such as array CGH are identifying increasing numbers of submicroscopic copy number alterations in mental retardation (MR). Interestingly, a diagnostic analysis of approximately 200 MR cases onto our tiling resolution BAC arrays detected two partially overlapping *de novo* microdeletions and one *de novo* microduplication in 2q23.1q23.2. Severe MR, postnatal growth retardation, microcephaly, coarse facies and epilepsy were noted in both microdeletion patients. The microduplication was detected in a severely mentally retarded boy with marked hypotonia.
The minimally affected region of 317 Kb encompasses two known genes: methyl-CpG binding domain protein 5 (*MBD5*), and enhancer of polycomb homolog 2 (*EPC2*). *MBD5* is an autosomal homologue of *methyl CpG binding protein 2* (*MeCP2*), implicated in Rett syndrome. Remarkably, some clinical overlap with Rett syndrome could be noted in our patients. In addition, duplication of *MeCP2* causes severe MR and progressive neurological symptoms, as was observed in our microduplication patient. Therefore, we performed mutation analysis as well as copy number analysis of *MBD5* and *EPC2* in 47 patients referred to our centre for *MeCP2* analysis, together with a 2q23 copy number analysis of 200 additional MR patients. No additional genetic alterations were detected.
In conclusion, 3 submicroscopic alterations at the 2q23 region have been identified in ~400 MR cases, pointing to a rather frequent microdeletion/duplication. The overlap in clinical features between the two microdeletion patients points to a new MR syndrome which needs to be further defined.
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P0190. Mental retardation and stiff thumbs: A further family with three sibs affected

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In 1983, Piussan et al described a family wherein females in 3 generations showed stiff thumbs, brachydactyly type A1 and mental retardation. Barber et al (1990) reported an isolated case of stiff thumbs and developmental delay which they thought represented the same disorder as that reported by Piussan. No further case had been reported in literature so far.
Here we describe a family with three affected male sibs. They all had uneventful pregnancy and delivery, regular height and weight growth, good general health. The eldest sib suffers from epilepsy by the age of 20 years. They show stiff thumbs, thenar hypoplasia, limited forearm pronosupination, short arched clavicle, plantar hyperkeratosis. We propose this family represents a further case of Piussan syndrome. Possible inheritance models will be discussed.
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P0191. Genetic causes of developmental delay and mental retardation

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Developmental Delay (DD)/Mental Retardation (MR) are frequently occurring disorders. Most studies that investigated the usefulness of diagnostic tests in patients with MR are performed in institutionalized patients with severe MR. This study examines the frequency and types of genetic defects among individuals undergoing neuropediatric and genetic evaluation because of DD and/or MR.
Cases were ascertained through the population-based Congenital Anomalies Registry of Navarra that monitors children with a birth defect, including DD/MR, whose mother lived in the province of Navarra at the time of diagnosis. All cases (248 males and 187 females) were evaluated on at least one occasion by a neupediatrician and/or a clinical geneticist. Conventional karyotype was performed on 390 (90%) cases; a total of 102 (23%) children were screening for fragile-X syndrome and 137 (31%) for subtelomeric rearrangements.
A specific genetic cause of DD/MR was found in 201 (46%) cases. Numerical aneuploidies were found in 121 (28%) children: 100 cases had Down syndrome, 14 were trisomy 18 and 7 showed a sexual chromosome aberration. Subtelomeric rearrangements were detected in 10 (2.3%) cases, 5 of whom had also other structural congenital defects. Eight children (1.8%) had a microdeletion syndrome and 8 (1.8%) carried other structural chromosomal anomaly. Single gene defects were observed in 47 individuals (10,8%), including seven cases (1,6%) with fragile-X-syndrome.
This study shows that subtelomeric rearrangements are the third most frequent cause of a DD/MR, especially if it is associated with structural defects.

P0192. Molecular-genetic cause of recessive congenital methemoglobinemia type I in Yakutia

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Recessive congenital methemoglobinemia (RCM) type I is a deficiency of nicotin amide adenine dinucleotide (NADH)-cytochrome b5

reductase (b5R). In Russia this disease widely distributed in Yakutia, where its frequency amount 1:5677. Our propose was to determine molecular-genetic cause of RCM type I in Yakutia and devise a simple method of diagnostics. We have considered that this disease had widely distribution in Yakutia due to local founder effect and chose 4 polymorphic markers flanking the human b5R gene *(DIA1,* 22q13.31*)* to check this idea. Most of affected probands were homozygous for all markers and have identical haplotype. All their healthy parents were heterozygous carriers for this haplotype; any other healthy relatives weren't homozygous for this haplotype. We suggested *DIA1* gene as candidate gene for RCM in Yakutia and sequencing all of 9 exons in one of our patients. We found out novel mutation CCG→CTG in homozygote, which led to amino acid substitution at position 269 (Pro269Leu) and created site for Alu1 ferment. After that we devised a method for simple detection of this mutation by RFLP-analysis which allowed detected this mutation in patients and their relatives, and reasonable for population investigation. Conclusion: We determined molecular-genetic cause of RCM type I in Yakutia and devised simple method for disease diagnostics in population.

P0193. Increased risk of migraine in Marfan Syndrome?

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Objective. To study the prevalence of migraine in Marfan syndrome. *Material and methods.* The patients were recruited from Landsforeningen for Marfan syndrome, a patient organization. A total of 46 persons were eligible for a validated semi-structured telephone interview by a physician trained in headache diagnostics. *Results.* The prevalence of migraine without aura was 13% among men and 40% among women. The prevalence of migraine with aura was 44% among men and 37% among women. The overall prevalence of migraine was 63% with an equal sex ratio. This corresponds with a 3.6- and 2.0-fold significant increased risk among men and women, respectively, compared with the general population.
Conclusion. The high prevalence and equal sex ratio of migraine is puzzling and likely to be secondary to Marfan syndrome. It might be associated with dural ectasia, since the prevalence of the dural ectasia is similar to that of migraine in Marfan syndrome.

P0194. Hepatocerebral mitochondrial DNA depletion caused by deoxyguanosine kinase (DGUOK) mutations

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Background: Autosomal recessive mutations in deoxyguanosine kinase (*DGUOK*) were repeatedly described in the hepatocerebral form of mitochondrial DNA (mtDNA) depletion (MDS).
Objectives: To describe the clinical spectrum of *DGUOK*-related MDS, we report 6 cases and summarize the literature.
Results: We identified pathogenic mutations in the *DGUOK* gene in 6 patients with the hepatocerebral form of mtDNA depletion. We describe the clinical, neuroradiological, histological and genetic features in these children. All patients showed severe hepatopathy, while involvement of other organs (skeletal muscle, brain) was variable. We identified five novel mutations (one of them in two patients) and two previously described mutations. Three different mutations affected the initiation methionine, suggesting a mutational hot spot. One of our patients underwent liver transplantation and pathology revealed, in addition to diffuse hepatopathy, a hepatocellular carcinoma, implying a possible link between mtDNA depletion and tumorigenesis.
Conclusions: We studied 12 cases with infantile hepatoencephalopathies and mtDNA depletion, and found pathogenic *DGUOK* mutations in 6, suggesting that this gene defect is a frequent but not exclusive cause of the hepatic form of mitochondrial DNA depletion syndrome.

P0195. Mutations in the ND5 subunit of complex I of the mtDNA are a frequent cause of OXPHOS disease

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Objective: To determine the frequency of mutations in the mitochondrial (MT)-ND5 gene in patients with OXPHOS disease. Background: Mutation detection in the mitochondrial genome is usually limited to common mutations and the tRNA genes. However, mutations in the ND subunits of complex I can be an important cause of OXPHOS disease. Methods: mutation screening of the mtDNA was performed by DHPLC-analysis of120 patients with a mitochondrial disorder. Heteroplasmy levels in different tissues were determined using PCR with fluorescently labelled primers and mutation specific restriction enzymes. Results: We found a MT-ND5 mutation in 3,3% of the patients. Two mutations were new and two have been previously reported. The 13513G>A mutation, associated with MELAS and MELAS/Leigh/Leber hereditary optic neuropathy overlap syndrome, was found in a relative low percentage in two patients, one with a Leigh and one with a MELAS phenotype. The 13042G>A, once detected in a patient with a MELAS/MERRF phenotype, was now found in a patient with a Leigh-like/NARP phenotype. A new mtDNA mutation (12622G>A) was identified in three brothers, all with infantile encephalopathy (Leigh syndrome) fatal within the first 15 days of life and a novel point mutation (13511A>T) occurred in a patient with a Leigh-like syndrome. Conclusions: Mutation screening of the mitochondrial ND5 gene is advised for routine diagnostics of patients with OXPHOS disease, especially MELAS- and Leigh-like patients.

P0196. Pathogenic mitochondrial mutations in Russian families with Leber's hereditary optic neurophany

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Leber's hereditary optic neuropathy (LHON) is a neuroophthalmologic disorder of mitochondrial origin that occurs all over the world. Three pathogenic, or primary, mtDNA mutations are responsible for over 90% of cases. DNA diagnostics was performed in a group of families with presumptive LHON. Primary mtDNA mutations were detected in 50 members (40 patients and 10 healthy relatives) of 36 unrelated families. Most of these families were Russians (26 families), the group included families of eight other ethnicities. The three mutations broke down as follows: G11778A in 80,6% (29 families), G3460A in 13,9% (5 families), T14484C in 5,6% (2 families). About 70% of cases are familial. The male/female ratio among 74 patients, genealogical data included, is 4,69. Three families are of particular interest: a G11778A mutation family with atypically high penetrance in females; a T14484C mutation family with concomitant diabetes mellitus in the proband and with a novel A9016G mutation in the ATPase 6 gene probably increasing the severeness of the disease. Finally, a G3460A mutation family occurred with homoplasmy in a young male proband and 40% heteroplasmy in his affected mother in whom the disease was induced by ethambutol and isoniazid.

P0197. A novel mitochondrial tRNAIle point mutation associated with chronic progressive external ophthalmoplegia

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Chronic progressive external ophthalmoplegia (CPEO) is a common clinical presentation of mitochondrial myopathy, either alone or associated with evidence of multiple system involvement, such as in Kearns-Sayre syndrome. Different mutations of mitochondrial DNA (mtDNA) have been correlated with isolated CPEO, namely large-scale rearrangements of mtDNA, either single or multiple deletions. These have usually been in sporadic forms or in cases with autosomal dominant or recessive inheritance. We report a sporadic case of

CPEO associated with ragged red fibers. Molecular genetic analysis did not reveal any rearrangements of the mtDNA. However, analysis of all mitochondrial tRNAs revealed a G to A transition at nt 4308 in the *tRNA^{Ile}* gene. The mutation, involving a highly conserved base-pair in the T-stem, was detected to a high level (>50%) in muscle, but not in blood. Furthermore, the mutation co-segregated with the phenotype, as the mutation was absent in blood and muscle form her healthy mother. The patient presented with enlarged mitochondria with deranged internal architecture and crystalline inclusions. Biochemical studies revealed reduced activities of complex I, III and IV in skeletal muscle. A possible link between CPEO with mtDNA deletions and CPEO with point mutations within the tRNA genes is the decrease in mitochondrial protein synthesis, responsible for the multiple partial defects of the respiratory chain enzymes.

P0198. MLPA analysis for specific syndromes reveals chromosomal imbalances in 5.4% of patients with dysmorphic features, idiopathic mental retardation and normal conventional karyotypes

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258 patients with dysmorphic features, mental retardation and normal conventional karyotypes were investigated with syndrome MLPA analysis (P064 probeset, MRC-Holland, for detection of (micro)deletions associated with 1p36-deletion-, Sotos-, Williams-, Prader Willi/Angelman-, Miller-Dieker-, Smith-Magenis-, and 22q11-deletion syndromes), subtelomeric MLPA (P019, P020 and P036 probesets, MRC-Holland), and HR-CGH.

Syndrome MLPA analysis revealed chromosomal imbalances in 5.4% of patients. The imbalances included deletions of 1p36, 5q35 (Sotos), 7q11.23 (Williams), 15q11.1q12 (Prader Willi/Angelman), 17p11.2 (Smith-Magenis) and 22q11. In addition **duplications** of 5q35 (NSD1 gene), 7q11.23, 17p13.3, 17p11.2 and 22q11 were detected. HR-CGH and subtelomeric MLPA revealed chromosomal imbalances in 11.2% and 5.4% of the patients respectively (Kirchhoff et al., AJMG 139A:232-233, 2005). Apart from revealing microdeletions associated with the syndromes mentioned above, syndrome MLPA seems to be a very useful approach to test for the reciprocal microduplications. These microduplications may be largely undetected due to technical difficulties in identifying them and to problems related to ascertainment of patients. Although several imbalances were detected by more than one analysis, syndrome MLPA, subtelomeric MLPA, and HR-CGH were complementary (in total, chromosomal imbalances were detected in 16.3% of patients). MLPA is a reliable and cost-effective method, which may be important when designing a clinical testing algorithm for mental retardation.

All MLPA data were analyzed by the use of normal reference data sets (details and free downloads of the software are available on www.chromosomelab.dk)

P0199. Coexistent mosaic monosomy 21 and fragile X syndrome in a mentally retarded male patient

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Fragile X syndrome (FXS) is a well-recognized mental retardation syndrome with characteristic facial features and behavioral phenotype. Monosomy 21 is a rare cytogenetic aberration for which clinical features were incompletely defined since full monosomy 21 is incompatible with life. A 5-year-old male patient with FXS and low-grade mosaicism for full monosomy 21 (46,XY [96%] / 45,XY,-21 [4%]) is presented. He had lack of speech with severely impaired social skills, hyperactivity, stereotypical hand movements, a special interest towards moving colorful items and a short attention span for other objects around. He had macrocephaly with a rather long face and a prominent occiput. He had a prominent midface with a beaked nose and retrognathia. His palpebral fissures were down-slanting and he had hypertelorism. Associating features include a rather long and smooth philtrum with a thin upper lip, prominent, cup-shaped, posteriorly rotated and low-set ears. No major malformations were present. Full monosomy in the aberrant cell line was proven by FISH with whole chromosome paint. Association of FXS with sex chromosome aberrations was reported several times, however, to the best of our knowledge, the present patient is the first FXS patient with an associating aberration of autosomal chromosomes. He contributes to the current knowledge on monosomy 21 phenotype, having the dysmorphic facial findings despite the concurrent phenotypic expression of the associating FXS. As a last conclusion, cytogenetic analysis must be done to all mentally retarded patients with minor dysmorphic features.

P0200. Investigation of Polymorphisms in Non-coding Region of Human Mitochondrial DNA in 31 Persian 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 positions 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.

P0201. Investigation of possible influence of Methyltetrahydrofolate reductase (MTHFR) polymorphisms on age at onset and severity of the Huntington disease

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Huntington disease (HD) is a genetic disorder of the central nervous system with symptoms usually appearing in adults within the third or fourth decade of life. Symptoms may include involuntary movements and loss of motor control. In addition, personality changes may occur, with loss of memory and decreased mental capacity. Huntington disease is inherited as an autosomal dominant condition. Increase in the number of CAG repeat, presented on chromosome 4p, causes HD. This sequence may be duplicated many times in individuals (up to 26 times) in the general population. Individuals with HD may have from 40 to over 100 repeated CAG segments.Since there is a hypothesis that the presence of some natural polymorphisms in some genes such as MTHFR could affect the Huntington disease, now we are aiming to investigate the possible role of these SNP in those clinically and genetically diagnosed affected patients. In addition we would like to establish a real time PCR assay for the diagnosis of the Huntington patients

P0202. Hereditary leiomyomatosis and FH mutations in Dutch families

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Multiple cutaneous and uterine leiomyomatosis (MCUL, OMIM

#150800) is a rare autosomal dominant condition, complicated by renal cell carcinoma in a subset of families (2-6%) (OMIM #605839). 46 UK, 35 North American and 3 Finnish families have been reported. In 80-100% of the families germline mutations in the fumarate hydratase (*FH*) gene on chromosome 1q42.3-43 have been identified.
We here report the findings of the first two leiomyomatosis families subjected to DNA analysis in the Netherlands. Two novel *FH* mutations were detected. In Family A, a missense *FH* mutation c.824G>A (p.Gly275Glu) co-segregated with clinically affected family members. All four affected females had uterine leiomyomas, two underwent hysterectomy at ages 27 and 32 years, respectively. One male affected family member had an incidentaloma in the right adrenal gland. Cutaneous signs were highly variable. In Family B a pathogenic c.1210G>T (p.Glu404X) *FH* mutation was found in the proband who presented with skin and uterine leiomyomas. As yet no renal cell cancer has been detected in either kindred.
In summary, the clinical signs of MCUL are very variable. The condition may be underdiagnosed in Dutch families. Early-onset severely symptomatic uterine leiomyomatosis is indicative of the syndrome.

P0203. Association of transforming growth factor-β1 gene polymorphisms with myocardial infarction in patients with angiographically proven coronary heart disease

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Objective: Transforming growth factor-β1 (TGF-β1) is a multifunctional cytokine that exhibits vasculoprotective properties. Production and plasma levels of TGF-β1 are influenced by polymorphisms in the TGF-β1 gene (*TGFB1*). We investigated whether the -509C/T (rs1800469), 868T/C (rs1982073), 913G/C (rs1800471), and 11929C/T (rs1800472) polymorphisms of *TGFB1* are associated with myocardial infarction.
Methods and Results: The study population consisted of 3657 patients with myocardial infarction (885 women and 2772 men) and 1211 control individuals (598 women and 613 men) with angiographically normal coronary arteries and without signs or symptoms of myocardial infarction. Polymorphism-related genotypes were determined with TaqMan assays and haplotypes were estimated from the genotype data. The -509C/T polymorphism and -509C/868T/913G/11929C (CTGC) haplotype were associated with myocardial infarction in men, independently from the potentially confounding factors age, arterial hypertension, hypercholesterolemia, cigarette smoking, and diabetes mellitus. Lower risks of myocardial infarction were observed among the carriers of the -509CC genotype (adjusted OR 0.49, 95% CI 0.27-0.87; *P*=0.014) and homozygous carriers of the CTGC haplotype (adjusted OR 0.61, 95% CI 0.38-0.98; *P*=0.042) than among the noncarriers of this genotype or haplotype. None of the genotypes (*P*≥0.37) or haplotypes (*P*≥0.35) was associated with myocardial infarction in women.
Conclusions: Positive association findings in this study suggest that *TGFB1* is a susceptibility locus for myocardial infarction.

P0204. Severe myotonia permanens revealed in neonatal period secondary to *de novo* new N1297K mutation in the skeletal muscle voltage gated sodium channel gene (*SCN4A*).

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Skeletal muscle channelopathies are disorders of muscle fibre membrane excitability and present clinically with varying combinations of periodic paralysis, myotonia, and paramyotonia. Mutations in the skeletal muscle voltage gated sodium channel gene (*SCN4A*) are associated with paramyotonia with a severe form called myotonia permanens, hyperkalemic periodic paralysis, and potassium-aggravated myotonia. The relation between genotype and phenotype is not always consistent. Here, we report on a 11-month-old infant referred to our unit after birth for multiple faints. She presented with daily recurrent episodes of severe desaturation with generalised muscular contractures and loss of visual contact. Family history was unremarkable. Clinical features included growth and weight retardation, axial hypotonia with peripheral hypertonia, facial dysmorphism with amimic facies and narrowed palpebral fissures, and muscular hypertrophy. There was no clinical myotonia. X-rays showed isolated hip dislocation. Electromyography revealed profuse myotonic discharges. Muscle biopsy was normal. After excluding high resolution chromosomal and telomeric abnormalities, Steinert dystrophic myotonia, Schwartz-Jampel syndrome, and hyperekplexia, DNA sequence analysis identified a *de novo* new heterozygous point mutation N1297K in *SCN4A* gene. The mutation leads to amino acid substitution in a highly conserved position within the loop between the third and fourth domains frequently implicated in pathogenic mutations. A link between cold temperature and myotonia was proved by immersion in cold water. Treatment with low dose mexiletine was moderately successful. In association with warm clothes, myotonic episodes became less frequent (two per month). This is the second report of a mutation in *SCN4A* gene in association with severe neonatal myotonia permanens.

P0205. Sleep disorders in the childhood-onset form of myotonic dystrophy

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Myotonic dystrophy type I (DM1) is an autosomal dominant trinucleotide repeat disorder that shows anticipation. Several forms exist which differ in age of onset and severity. Excessive daytime sleepiness (EDS) is frequent and well documented in adults and it is also a prevalent complaint in the population of patients with congenital and childhood-onset forms of DM1 but its objective assessment remains elusive. It could likely contribute to learning disabilities and if so, a treatment could be proposed.
To assess more precisely the sleep disorders in these children, we have led a prospective study in 21 children affected by a childhood-onset form (congenital form were excluded), from 6 to 19 years old. We carried out, for all of them, a clinical examination, a sleep recording and multiple sleep latency tests (MSLT). They also completed a sleepiness self evaluation scale (Lecendreux scale).
We found sleep troubles in 66% of patients. 29% present a sleep apnea syndrome and 38% present a periodic limb movements syndrome (PLMS).
To our knowledge, it is the first time that PLMS is reported in children with DM1. We haven't found any correlation between the existence of sleep trouble and clinical or molecular criteria except for a higher MDRS (Myotonic Disability Rating Scale) and a more frequently reported fatigue.
This study shows the high frequency and the different origins of sleep troubles in children affected by DM1 and allows us to consider specific treatment for each of its origins, that could help to improve the management of these children.

P0206. Neonatal diabetes mellitus associated with duplication (6)(q23.3q24.2)

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We report a female who presented at 8 days of age with weight loss, dehydration and a glucose of 42 µmol/L. She was born at 36 weeks gestation with symmetric growth parameters at the 3rd percentile, minor dysmorphic features, and an umbilical hernia. At 4 months of age she was weaned from insulin and has had no recurrence at one year, consistent with apparent remission. Cytogenetic analysis identified a de novo duplication of chromosome 6 with a karyotype of 46, XX, dup(6)(q23.3q24.2).ish dup (6q)(wcp6+).
Neonatal diabetes mellitus (NDM) is a rare condition with an estimated incidence of 1 in 400,000. NDM is divided into transient neonatal diabetes (TNDM) and permanent neonatal diabetes (PNDM). TNDM

accounts for 50-60% of cases and has a typical pattern of neonatal presentation with apparent remission, usually in the first six months of life, as well as a predisposition to later onset type 2 diabetes. Paternal duplications involving 6q24, paternal uniparental isodisomy, and methylation defects at a CpG island overlapping exon 1 of *ZAC/HYMAI* have all been implicated in the pathogenesis of transient neonatal diabetes. A literature review revealed two other cases with a cytogenetically visible duplication of chromosome 6 in association with TNDM.

P0207. Genotypic and phenotypic spectrum of *PANK2* mutations in patients with NBIA

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Neurodegeneration with brain iron accumulation (NBIA) is a group of disorders characterized by MRI changes in basal ganglia. A distinguished group of NBIA patients show the typical eye-of-the-tiger sign in MRI. Both missense and nonsense mutations have been found in such patients in a gene encoding the mitochondrial pantothenate kinase (PANK2), which is predicted to catalyse the first step of CoA-biosynthesis.

We completed a mutation screen in 91 patients with the diagnosis NBIA based on clinical findings and radiological imaging. The entire coding region of the *PANK2* gene (20p12.3) was investigated for point mutations and deletions. We uncovered both mutant alleles in 52 patients. Deletions accounted for 4% of mutated alleles. Patients with two loss-of-function alleles (n=13) displayed symptoms always at an early stage of live. In the presence of missense mutations (n=39) the age of onset correlated with residual activity of the pantothenate kinase. Progression of disease measured by loss of ambulation was variable in both groups. We did not observe a strict correlation between the eye-of-the-tiger sign and *PANK2* mutations. No *PANK2* mutation was identified in 39 patients. Clinical features of both groups were assessed and analyzed for similarities and discrepancies.

Conclusions: Deletion screening of PANK2 should be part of the diagnostic spectrum. Other factors than enzymatic residual activity are determining the course of disease. There are strong arguments in favour of locus heterogeneity.

P0208. Molecular testing in Neurofibromatosis type 1 (NF1): mutational spectrum, patterns of recurrence and correlation with clinical features in Italy

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Neurofibromatosis type 1 (NF1) is a common autosomal dominant disorder in humans affecting 1 in 3500 individuals. In this work we review the mutational reports on Denaturing high performance liquid chromatography (DHPLC) analysis of the NF1 gene published until now and the NF1 clinical and molecular findings in the Italian population by reporting our experience with multiple screening techniques in the analysis of the whole NF1 coding region in a panel of 468 consecutive NF1 patients from 8 Italian centres catering with NF1. The study of 468 NF1 patients has been performed using multiple screening techniques, viz. protein truncation test (PTT), heteroduplex analysis (HA), fluorescence in situ hybridisation (FISH), Southern blotting, cytogenetic analysis, and DHPLC. Particularly, our study performed by DHPLC on 374 patients revealed 187 novel mutations with a mutation detection rate (in the northern eastern Italian regions and in Sicily) of 83% (i.e., the highest so far reported). Seventy-four of the 374 unrelated NF1 patients harboured 35 different recurrent mutations. Genotype-phenotype analysis revealed a higher rate (75%) of missense mutations (vs. other mutations) in patients harbouring non-optic pathway cerebral gliomas and a higher degree of severity of pigmentation anomalies in the group of patients with missense mutations (89%). We also summarised the Italian NF1 microdeletion spectrum (n = 18), which by refined FISH characterisation could be classified as typical (n= 14) and atypical (n=4). This work confirms that DHPLC analysis for routine molecular diagnosis in NF1 is a simple and efficient methodology with a high mutation detection rate.

P0209. Clinical, Molecular and Immunohistochemistry Investigations of Neuromuscular Disorders in Iranian Populations

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Introduction: Neuromuscular disorders are the most common progressive group of heterogeneous disorders and there are considerable genetic heterogeneity in these group of disorders that over hundreds genes have been involved in neuromuscular disorders. The major symptoms of neuromuscular disorders are generalized muscle weakness and wasting, muscular atrophy, extraocular ophthalmoplagia, respiratory, cardiac, and other smooth muscles involvement. These disorders classified as follow, myopathies (muscular dystrophies); neuromuscular Junction disorders (congenital myasthenic syndromes); neuropathies (Charcot- Marie -Tooth); motor neuron disorder (Spinal Muscular Atrophy).The aim of this study was classification of neuromuscular disorders based on clinical, molecular and immunohistochemistry (IHC) techniques in Iranian patients referred to Genetic Research Center during one year.

Result: We found 82 patients with Myotonic Dystrophy, 19 Duchenne/Becker Muscular dystroophy (DMD/BMD), 21 Limb Girdle Muscular Dystrophy (LGMD), 3 Fascioscapulohumeral Muscular Dystrophy (FSHD), 2 Congenital Myasthenic Syndromes (CMS), 6 Congenital Muscular Dystrophy (CMD), 10 Spinal Muscular Atrophy (SMA) based on clinical examination, electromyography (EMG) and muscle enzymes.

Conclusion: Therefore we must investigate the rest of the patients with DM diagnosis for DM2 and also the rest of patients with clinical symptoms of LGMD that they had normal sarcoglycan and dysferlin proteins in IHC. We need more analysis by using multiplex western blot technique to detect calpain, dysferlin, and sarcoglycan proteins in LGMD patients. More investigations are mandatory for finding of other types of neuromuscular disorders.

P0210. Identification of a novel mutation in the *PTCH* gene in a Korean family with nevoid basal cell carcinoma syndrome

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The Nevoid Basal Cell Carcinoma Syndrome (NBCCS) is an autosomal dominant disorder characterized by the development of

multiple jaw keratocysts, basal cell carcinomas, skeletal anomalies, ectopic calcification and palmoplatar pits. Mutations in the *PTCH* gene on the chromosome band 9q22.3, the homologue of the *Drosophila PATCHED* gene, have been identified to be the underlying genetic defects of the syndrome. We found a Korean family with NBCCS and identified a novel mutation in the *PTCH* gene. The proband, a 14-year-old girl, and her father, 42-year-old, were diagnosed as NBCCS based on clinical diagnostic criteria. To confirm *PTCH* gene defects, we performed mutation analysis for *PTCH* gene on the proband and her family members after we had obtained informed consent. Genomic DNA was isolated from peripheral blood leukocytes and all 23 coding exons of the *PTCH* gene were amplified by PCR and cycle sequencing was performed on the ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The proband was revealed to have a novel insertion mutation in exon 6 of the *PTCH* gene (c.817_818insT; Y273LfsX12), and further analysis of her family members demonstrated that only her father had the same mutation. This is the first report of genetically confirmed case of NBCCS in Korea.

P0211. Nevus of Ota associated with multiple Mongolian spots in infant - case report

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Mongolian spot is a hereditary developmental condition caused by entrapment of melanocytes in the dermis during their migration from the neural crest into the epidermis. A child may have one or several on the lower back They are more common in dark-skinned infants and, in some cases, involve unusual sites. Nevus of Ota is a permanent unilateral congenital/aquired blue/gray patch on the face and it may involve the ocular surface.

We present a five-months old dark-skinned female admitted in our clinic for pneumonia and seizures. She is the third child of an young healthy couple. The pregnancy was not followed up and she was delivered full term, with a weight of 3880 g and Apgar score 9. Girl's growth and development were normal.

By clinical examination we noticed multiple bluish spots involving large skin areas: the right side of the face, shoulders, back, presacral area and buttocks. Right eye's bulbar conjunctiva was also involved. The patchs and eye hyperpigmentation are congenital and they have not changed since birth. Ophtalmologic examination identified melanocytosis of the entire uveal tract and normal intraocular pressure. MRI of the brain was normal.

Conclusions: There is a moderate case of nevus of Ota with ocular melanosis. Ophtalmologic and dermatologic follow-up care is necessary.

P0212. New MCA/MR syndrome; Distinct facial features with cerebellar and genital hypoplasia

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We here report three children with mental/motor retardation, distinct facial dysmorphism and hypoplastic genitalia. Two are siblings, a nine year old female and a four year old male, born to a first degree and the third, a ten year old male, born to a second degree cousin marriage. All three have microcephaly, psychomotor retardation, distinct facial features and hypoplastic genitalia. Dysmorphic facial features are low frontal hairline, arched eyebrows with medial flare, down slanting palpebral fissures, epicanthic folds, long eyelashes, and prominent nasal root. Hypoplastic genitalia is present with aplastic scrotum, undescended testis and hypospadias in males and agenesis of labium majus/minus in the female. The ethiological work up revealed cerebellar hypoplasia in cranial MRI and EEG abnormalities in all. Karyotype analysis and metabolic screening were normal.

Similar phenotype observed in the siblings and consanguinity in both families are suggestive for autosomal recessive inheritance. The databases were screened in order to define a MCA/MR syndrome with autosomal recessive inheritance that is compatible with the findings of our patients. Although several features of MacDermot-Winter syndrome were overlapping with our patients' findings; IUGR, feeding difficulties and short life span were not observed in our cases. Many other MCA/MR syndromes with hypoplastic cerebellum and genitalia are known. However, our cases do differ with the existence of distinct facial features and agenesis of labioscrotal folds which is at the severe end of the hypoplastic genitalia spectrum.

The clinical, cytogenetical, molecular cytogenetical and biochemical findings of this previously unreported MCA/MR syndrome will be presented.

P0213. Nijmegen Brekage Syndrome with Myelodysplasia

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Nijmegen Breakage Syndrome (NBS) is an autosomal recessive chromosomal instability syndrome. The main characteristics of NBS include short stature, progressive microcephaly,
immunodeficiency and predisposition to malignant diseases (especially lymphomas), microcephaly, delayed development and characteristic facial features. Spontaneous chromosomal instability, cellular instability, and clonal occurrence of rearrangements involving chromosomes 7 and 14 are present. Mutations of the NBS1 gene are detected in nearly all patients.

Case report: We described a 8 years old boy presented with low-set ears, prominent nasal bridge, micrognathia and left skin syndactyly (III-IV toe), homozygous for the 5- bp deletion (657del 5) in exon 6 of the NBS1 gene. In the described patient, standard GTG-banded peripheral blood chromosomal analysis showed the 46, XY karyotype, with a high level of spontaneous chromosome breakage. Clinical examination showed continuing evidence of failure to thrive, extreme microcephaly, hypogammaglobulinaemia, frequent respiratory infections, peripheral blood cytopenia. He developed progressive bone marrow failure and was transiently treated with substitution of immunoglobulines, different antibiotics, G-CSF and erythropoetin.

P0214. The NOGGIN mutation M190V leads to the phenotype of facio-audio-symphalangism syndrome in a family

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Heterozygous mutations in the *NOGGIN* gene are known to cause different autosomal dominant inherited disorders such as isolated proximal symphalangism (OMIM #185800), tarsal-carpal coalition syndrome (OMIM #186570), and multiple synostoses syndrome (OMIM #186500) whereas the facio-audio-symphalangism syndrome is listed in the OMIM database as a synonyme for multiple synostosis syndrome. NOGGIN is secreted as a homodimer that binds to bone morphogenetic proteins (BMPs) and acts in an antagonistic way to BMP signalling. Through this inhibiting effect on the BMP pathway, a proper development of bones and joints as well as frontonasal and ocular structures is getting disturbed demonstrated by different studies. We report on three affected family members with typical features of facio-audio-symphalangism syndrome. To a variable degree, the affected persons had a distinctive facies with a particular nose, conductive hearing loss due to stapes ankylosis, hyperopia, proximal symphalangism and stiffened or broad thumbs. In this family, a novel missense mutation M190V lying in a highly conserved C-terminal region of NOGGIN was identified. This mutation presumably disrupts a cysteine-knot motif consisting of nine cystein residues located in this region being important for disulfide-bond formation of NOGGIN homodimers. In addition, a specific genotype-phenotype correlation of NOGGIN mutations is not predictable at present. Also recent reports show that the phenotypic spectrum of NOGGIN mutations is presumably broader than expected in the recent years.

P0215. Proximal symphalangism - follow-up

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Proximal symphalangism is a rare AD disorder defined by fusion of the proximal interphalangeal joints, humeroradial fusion, tarsal and carpal coalition, absence of interphalangeal folds and conductive hearing loss.

It is produced by mutations in NOG gene encoding Noggin protein.
We describe a family in order to illustrate the clinical features, to present the evolution (8 years) of the clinical picture and to discuss some particularities. The proband (8 years old girl) presents: bilateral elbow ankilosis and limited movement of proximal interphalangeal joints. Lower limbs: muscular hypotrophy, bilateral pes supinatus, irregular toe implantation, limited toe movements. Intellectual development and hearing are normal. Her father and his sister present the same features. Their mother is phenotypically normal. Radiologic examination is similar for all of them: upper limbs- humeroradial fusion with loss of articular space, carpal bones fusion, hypoplastic second phalanx fused to proximal phalanx (fingers 2-5); lower limbs- tarsal bones fusion, calcaneus fused to the tarsal block, fusion of phalanges 4th toe (toes 3-5 in the aunt). Differential diagnosis was done with: Facio-audio-symphalangism syndrome, Humero-radial synostosis and Tarsal-carpal-coalition syndrome. Clinical features were constant in the father and his sister; in the daughter bone fusion and muscle hypotrophy were delayed with physical therapy. The particularity of this family is that the father and his sister probably represent the consequence of gonadal mosaicism (they resulted from 2 different marriages of a phenotypically normal woman). In conclusion, we present a family with proximal symphalangism in order to illustrate the features, the evolution and particularities.

P0216. Rhabdomyosarcoma in a patient with Noonan syndrome phenotype

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An increased risk of malignancy has been reported in patients with Noonan syndrome (NS), an autosomal dominant disorder with variable expression and incomplete penetrance. The SH2 domain-containing protein-tyrosine phosphatase (SHP2), encoded by the responsible gene (PTPN11), is required for normal development and is an essential component of signaling pathways initiated by growth factors and cytokines, in many of which SHP2 possibly acts upstream of Ras oncogene and interferes in tumorigenesis. We report a patient with short stature, tall forehead, downslanting palpebral fissures, hypertelorism, depressed nose root, webbed neck, pectus excavatum and developmental delay. He also had a history of surgical reconstruction of pulmonary stenosis along with hypertrophic cardiomyopathy and posthemorrhagic hydrocephalus after perinatal distress. It is mentioned that our proband did not present any of the cardinal manifestations of Costello syndrome (papillomata, acanthosis nigricans, loose skin, deep palmar creases), which shows phenotypic overlap with NS. At the age of 5 years he presented with recurrent abdominal pain and constipation. Laboratory evaluation with U/S and CT-scan demonstrated the presence of an abdominal mass. A total resection of the mass and consequent histology revealed embryonal rhabdomyosarcoma. Rhabdomyosarcoma is a rare tumor in children with NS and to the best of our knowledge only four cases have been reported so far, while in Costello syndrome, the frequency of rhabdomyosarcoma is approximately 17%. The presentation underlines the importance of frequent follow up of patients with NS phenotype, since the incidence of various malignancies is small but existing.

P0217. Two novel PTPN11 mutations in familial and sporadic Noonan syndrome

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Noonan syndrome (NS, OMIM 163950) is an autosomal dominant developmental disorder characterized by facial dysmorphisms, skeletal anomalies, cardiovascular abnormalities, and haematological disorders . The phenotype is extremely variable and includes webbed neck, chest deformity, mild mental retardation, cryptorchidism, feeding difficulties, bleeding diathesis, myeloproliferative disorders, and lymphatic dysplasias. This condition is relatively common, with a prevalence at birth of 1:1000-2500 live births. Missense mutations in the PTPN11 gene on chromosome 12 (12q24) account for approximately 50% of NS cases. The gene encodes a non-receptor protein tyrosine phosphatase (PTP) SHP-2, with NS mutations resulting in gain of function. A significantly higher prevalence of mutations is described in familial cases than in sporadic ones. Genotype-phenotype correlations demonstrate that pulmonary stenosis is associated with PTPN11 mutations, whereas hypertrophic cardiomyopathy is associated to mutation-negative cases. In this study, we have analysed 27 patients with NS clinical phenotype. Molecular lesions have been identified in 8 of them, presenting typical clinical features associated to pulmonary valve stenosis. A familial novel mutation, Leu261His, has been identified in two sisters, and a Leu262Arg missense mutation has been observed in a sporadic case.

P0218. The recently reported rare A172G mutation of PTPN 11 gene seems to provoke a mild Noonan Syndrome phenotype

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Background: Noonan syndrome (NS) (OMIM 163950) is an autosomal dominant developmental disorder characterized by typical facial dysmorphism, growth retardation and variable congenital heart defects. In unrelated individuals with sporadic or familial NS, heterozygous missense point mutations in the gene PTPN11 (OMIM 176876) have been confirmed, with a clustering of mutations in exons 3 and 8, the mutation A 922 G (Asn 308 Asp) accounting for nearly 25% of cases. Patient and methods: We report a 7 years old boy with short stature and some other clinical features of Noonan syndrome, who has been investigated by molecular analysis for the presence of mutations in the PTPN11 gene. Results: The de novo mutation A 172 G in the exon 3 of the PTPN 11 gene, predicting an Asn58Asp substitution, has been found. To the best of our knowledge this specific mutation has only been described once before, but this is the first report of detailed clinical data suggesting a mild phenotype. Conclusions: Detailed clinical phenotype in every patient with features of Noonan syndrome and molecular identification of PTPN11 gene mutation may permit a better phenotype-genotype correlation.

P0219. Antenatal presentation of the Oculo-Auriculo-Vertebral Spectrum (OAVS)

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Oculo-auriculo-vertebral spectrum (OAVS or hemifacial microsomia) is a relatively common disorder affecting ~1 in 5600 live birth and results from a defect of blastogenesis involving primarily aural, oral and mandibular development. Clinically, OAVS ranges from microtia or auricular or preauricular abnormalities with or without mandibular hypoplasia, to a complex phenotype with skeletal, cardiac, renal, pulmonary, and CNS manifestations. Vertebral anomalies and epibulbar dermoids, when present, delineates the so-called Goldenhar syndrome. While diagnosis and management of OAVS are well established after birth, its antenatal pattern of malformations is still poorly delineated.
We describe a fetus with abnormal ultrasound imaging at 20 weeks showing hydrocephalus and radial aplasia. Post-mortem examination followed pregnancy termination and confirmed the diagnosis of OAVS.
To delineate the spectrum of prenatal features of OAVS, we review 20 published fetuses showing abnormal US/MRI imaging. Cephalic abnormalities were found in 52.4% (i.e. micro/anophthalmia, ear anomalies, hemifacial microsomia and facial cleft). CNS defects

occurred in 47.6% (i.e. hydrocephalus, occipital encephalocele, cerebellar hemisphere/vermis hypoplasia and lipoma of the corpus callosum), together with abnormal amniotic fluid volume (AFV), either poly- or oligohydramnios. Nineteen percent had congenital heart disease. Additional findings included hydroureteronephrosis, radial aplasia, lung and kidney agenesis.
Prenatal features of OAVS are highly heterogeneous. Facial asymmetric lesions (i.e. hemifacial microsomia, ipsilateral micro/anophthalmia, malformed ear) represent a recurrent pattern of anomalies. The association of CNS (particularly hydrocephalus) and AFV abnormalities is also common. Detection of heart, kidney, lung and skeletal anomalies may further support the prenatal recognition of OAVS.

P0220. Blepharophimosis-mental retardation (BMR) syndromes. A proposed clinical classification of the so-called ohdo syndrome, and delineation of two new syndromes, one x-linked and one recessive

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We report 11 patients from eight families with a blepharophimosis and mental retardation syndrome (BMRS). Five sporadic patients have the Gestalt of Ohdo syndrome, associated in two of them with congenital hypothyroidism (thus also compatible with a diagnosis of Young-Simpson syndrome). In two affected sibs, compensated hypothyroidism was demonstrated. In another family, an affected boy was born to the unaffected sister of a previously reported patient. Finally, in the last sibship, two affected boys further had severe microcephaly and neurological anomalies. A definite clinical and etiologic classification of BMRS has still to be invented, but closer phenotypic analysis should lead to a more analytic appraisal of the BMRS phenotype. We suggest to drop the term “Ohdo syndrome”, and to classify ,BMRS in at least five groups : 1) del(3p) syndrome, (possibly overlooked in older reports); 2) BMRS, Ohdo type, limited to the original patients of Ohdo; 3) BMRS SBBYS (Say-Barber/Biesecker/Young-Simpson) type, with distinctive dysmorphism and inconstant anomalies including heart defect, optic atrophy, deafness, hypoplastic teeth, cleft palate, joint limitations, and hypothyroidism. BMRS type SBBYS probably is an etiologically heterogeneous phenotype, as AD and AR forms exist; 4) BMRS, new probably XLR type (Maat-Kievit-Brunner type) with coarse, triangular face; 5) BMRS new probably recessive type (Verloes type) with severe microcephaly, hypsarrhythmia, adducted thumbs, cleft palate and abnormal genitalia. Independent of nosological considerations, our paper illustrates probable AR and XLR inheritances in patients with Ohdo syndrome.

P0221. An oligoarray CGH analysis on a patient with multiple congenital abnormalities

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An oligobased CGH microarray analysis was performed on a patient with a growth retardation of prenatal onset, several congenital abnormalities, and normal mental capacity. The adult heigth of this female patient is 144.5 cm (- 3 SD). She has a congenital heart defect presenting as a prolapse of the mitral and tricusbidal valves. The patient has been operated for scoliosis three times, and she also has joint laxity. She has a bilateral sensorineural hearing defect, and has been operated for bilateral strabismus. Her puberty has been delayed. The karyotype of the patient is normal 46, XX. The arrayCGH analysis using Agilent 44 kb oligoarray revealed an amplification of one gene in chromosome 18q22.1, and a small deletion and a amplification in chromosome 21q21. A polymorphic amplification was seen in the X-chromosome. The symptoms of the patient and the amplificated and deleted sections of the genome are discussed in detail in this poster.

P0222. An autosomal recessive phenocopy of Otopalatodigital syndrome type 2.

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Otopalatodigital syndrome type 2 (OPD2; OMIM 304120) is inherited as an X-linked condition and is characterised by a severe osteodysplasia and multiple extraskeletal congenital anomalies in affected males. OPD2 is allelic to OPD1 (OMIM 311300), Melnick-Needles syndrome (OMIM 309350) and frontometaphyseal dysplasia (OMIM 305620), with clustered missense mutations found in the gene encoding the cytoskeletal protein filamin A (*FLNA*; Xq28) in all four conditions. We describe a total 5 patients (2 males, 3 females) from 3 consanguineous families with a phenotype very similar to that observed in male individuals with OPD2. The first family consisted of a sib pair with a severe phenotype including a severe skeletal dysplasia and encephalocele, born to first cousin parents. The other two families are slightly less severely affected (2 sisters and a singleton male both born to parents who were first cousins). The *FLNA* gene was screened for mutations in all three instances but no mutations were identified.
The combination of a phenotype affecting both sexes to the same extent in the context of parental consanguinity and no mutation identifiable in FLNA, strongly suggests an autosomal recessive trait. This observation is consistent with the previously reported failure to identify *FLNA* mutations in a minority of unrelated sporadic OPD2 cases. The identification of an AR form of OPD2 has major implications for genetic counseling in all OPD2 cases of either sex who do not have a *FLNA* mutation identified. A genome wide search for the causative gene in these three families is currently underway.

P0223. Oro-Dental Abnormalities in Patients with Mental Retardation

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The aim of our present work is to investigate the prevalence of oral and dental abnormalities in patients with mental retardation. Also to delineate the most common patterns of presentation associated with oro-dental abnormalities. The present study included 132 cases with mental subnormality, who attended the human genetics clinic; National research center (N.R.C). A full history was taken for all the patients. Thorough oro-dental examination and IQ assessmentwere performed. Chromosomal studies and metabolic screening were done when needed.
The studied cases were divided into four groups according to the etiology of mental retardation: chromosomal, single gene defect, multifactorial and unclassified groups. The chromosomal group showed the highest percentage of oral region abnormalities (97%) and mouth abnormalities (81%), followed by the single gene defect 89% and 78% and the multifactorial group 80% and 81%.
The chromosomal group showed tongue abnormalities in 62% and palatal abnormalities in 63%. The single gene defect and the multifactorial groups showed maxillary abnormalities in 19%. As most cases with chromosomal abnormalities have severe to profound mental retardation we concluded that oro-dental abnormalities are more frequent in patients with severe and profound mental retardation, We recommend medical dental services and care for the affected cases since there is no national oral disease prevalence data with patients with mental retardation and developmental disability

P0224. Diagnostic issues in patients with oro-facial clefts

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Aim. To present 4 infants with different oro-facial clefts in which several diagnostic issues emerged after clinical examination and genetical investigations.

Material and Method. 4 infants (3 boys and 1 girl) with oro-facial clefts admitted in our clinic during January 2005-January 2006 were assessed for other malformations: complete clinical examination, cardiac non-invasive explorations, abdominal ultrasonography, neurological examination, laboratory investigations and karyotype.

Results. Two of them presented palate cleft without other malformations and the other 2 lip and palate clefts with associated congenital heart defects: VSD - 1 case and Tetralogy of Fallot - 1 case. All 4 presented failure to thrive, mild developmental delay, facial dysmorphic features, hypotonia and varus equin. The patient with associated Fallot disease also presented cryptorchidism and genital microsomia. Previous to karyotype investigation the 2 patients with associated cardiac defects were classified as velo-cardio-facial syndromes. The karyotype of the patient with Tetralogy of Fallot showed: unexpected 22 monosomy with unbalanced translocation t(15;22): 45, XY-22t (15,22)(q26.2;q12.2) 15q+. This karyotype was inconsistent with Shprinzen syndrome diagnosis. The infant with associated VSD was admitted in January 2006 and the karyotype is currently pending. For this time however he is considered as velo-cardio-facial syndrome, awaiting karyotype confirmation. The 2 infants without cardiac defects presented normal karyotype and are classified as oro-facial clefts.

Conclusions. Patients with oro-facial clefts must undergo complex investigations in other to rule out other possible malformations and to achieve an accurate diagnosis. Genetic tests are mandatory allowing proper classification of syndromes and sometimes discovering unexpected mutations.

P0225. Malignant osteopetrosis - a rare skeletal dysplasia mimicking progressive metabolic disease

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Infantile osteopetrosis is a rare lethal condition estimated to occur in 1: 200.0000 birth. This disorder is characterised by the inability to resorb bone, leading to increased density of nearly all bones, early onset of bone marrow failure, visual and hearing loss, psychomotor retardation, seizures and death in the first few years related to recurrent infections. We report on two affected children of consanguineous parents from Turkey. The older child presented in the first weeks of life with anemia, thrombopenia and hepatosplenomegaly, early visual impairment, severe hypotonia and nearly drug-resistant seizures, and died of persistent febrile infections at the age of 10 months. The development of the second child has been highly reminiscent of the sisters' course so far. He also revealed transfusion-dependent anemia as a newborn, and later on a similar loss of nerval functions. Because of hepatosplenomegaly and the involvement of grey matter dysfunction, a progredient neurometabolic disturbance was suggested first, which was ruled out by exhaustive biochemical studies. Interestingly, CK-BB isoenzyme was elevated 20fold over normal. Detailed differential diagnosis of the ineffective extramedullary hematopoesis established finally the diagnosis by the pathognomonic X-ray findings. The patient is now one year of age. Molecular studies are currently on the way to identify the underlying mutation in the two most probable candidate genes TCIRG1 and CLCN7. Both gene products are known to be involved in proton transport prerequisite for adequate lysosomal degradation and bone resorption by osteoclasts.

P0226. Estimating the gene frequency of malignant autosomal recessive osteopetrosis in Volga-Ural region populations of Russia and dating the mutation in Chuvashiya.

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Malignant autosomal recessive osteopetrosis (OPTB1) is included in group of inherited sclerosing osteochondrodysplasias and defined by general increased bone density. Early manifestation, progressing clinical current and early lethality characterize OPTB1. It is a rare disease in the world but the disorder has unusually high frequency in Chuvash republic of Russia (1 : 3900 newborns). All OPTB1 cases here is caused by single mutation c.807+5G>A in *TCIRG1* gene encoding one of functional markers of osteoclasts. In the previous research we have shown that the mutation was diffusion here by founder effect. In this work we have evaluated the gene frequency in Chuvash and else in two populations of Volga-Ural region (Mari and Udmurt). Allelic frequency of the mutation appeared is equal 1.7% in Chuvashiya (calculated frequency of disease 1 : 3500 newborns) and 0.8% in Mari (1 : 14 000 newborns). The haplotype analysis has shown that OPTB1 cases in Chuvashians and Marians originate from single mutational event. The mutation was not revealed in 396 Udmurts. Also we have calculated date of diffusion of the mutation in Chuvashians. For this purpose we used both the formula applied by other scientists and the approach offered by us. Our approach appeared giving more exact estimation and has allowed to estimate dating of the mutation as approximately 480 years ago. The recent "bottle neck" of this recessive mutation and its high current frequency suggest that the Chuvash population descends from a limited group of founders with its subsequent rapid growth.

P0227. Oto-Facio-Cervical syndrome and Branchio-Oto-Renal syndrome are variable clinical presentations of the same entity.

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Oto-Facio-Cervical syndrome (OFC, OMIM 166780) is characterised by an elongated face, preauricular pits, conductive hearing loss, lateral cervical fistulae, sloping shoulders, winged scapulae, and mild mental retardation. Branchio-Oto-Renal syndrome (BOR, OMIM 113650) is characterised by conductive and sensorineural hearing loss, external ear abnormalities, ear pits or tags, branchial cleft sinus or fistula, and variable renal abnormalities such as duplication of the collective system, and unilateral or bilateral renal aplasia. BOR is caused by mutations in the drosophila absent eyes (EYA1) gene on 8q13.3. Both conditions are considered separate in the literature. Penni et al (1992) however comments on shoulder abnormalities in BOR, whereas Rickard et al (2001) find an EYA1 abnormality in a patient with OFC. We describe a sporadic female patient with OFC syndrome and in addition unilateral renal agenesis as in BOR. EYA1 mutation analysis was normal. A second patient from a classical BOR family is described. In this family, an EYA1 mutation was found. This patient has small sloping shoulders as in OFC. We conclude that our cases are further proof that OFC and BOR represent the same genetic entity.

P0228. The outcome of ovarian cancer screening in a consecutive series of BRCA1/2 mutation carriers

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Introduction: BRCA1/2 mutation carriers have been offered gynecological screening aiming to detect cancer at an early stage or prophylactic bilateral (salpingo-)oophorectomy aiming to reduce the incidence of ovarian cancer risk. The purpose of the study was to investigate efficacy of gynecological screening.

Material and Methods: In this multi-center study we examined 888 BRCA1/2 mutation carriers who started gynecological screening (annually serum CA125 measurements and transvaginal ultrasonography) between 1993-2005. The Standardized Incidence Ratio (SIR) of detecting ovarian tumors during screening was calculated

based on age-, mutation-specific incidence rates.
Results: At the first screening visit four stage III ovarian cancers were diagnosed. In the remaining 884 women six screen-detected (one stage II, four stage III and one stage IV) and four interval ovarian cancers (one stage II, one stage III and two stage IV) were diagnosed during follow-up. The SIR of observed (10) versus expected (6.6) ovarian cancers was 1.5 (95%CI: 0.7-2.8). In total, 434 (87%) women were screened according to well-defined guidelines, during 606 women-years. Among these women, five ovarian tumors were diagnosed (one stage II, two stage III and two stage IV) giving a SIR of 1.7 (95%CI: 0.6 - 4.0).
Conclusion: Screening resulted in, though not statistical significantly, more ovarian cancers diagnosed than could be expected. Given the advanced stages at diagnosis, it remains to be investigated whether this will affect mortality from ovarian cancer in BRCA1/2 mutation carriers.

P0229. Paget's disease of bone in the French population: novel *SQSTM1* mutations and genotype phenotype correlation

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Introduction: Paget's disease of bone (PDB) is a frequent chronic disease of the skeleton. PDB often segregates with an autosomal dominant pattern of inheritance and incomplete penetrance. Genetic heterogeneity has been demonstrated. At the chromosome 5 locus, a recurrent mutation (P392L) of the *sequestosome 1 (SQSTM1)* gene was identified and several other mutations of this gene were described in PDB patients. All these mutations affect the highly conserved ubiquitin-associated domain (UBA) of the p62 protein. This study aims at evaluating the frequency of the *SQSTM1* mutations in the French PBD patients and to search for genotype phenotype correlations.
Patients and methods: 94 unrelated French PDB patients underwent genetic testing, relying on a sequencing of the 7 and 8 exon. For the genotype phenotype correlations study, an ANOVA analysis and chi-square test were performed.
Results: Mutations of *SQSTM1* gene were identified in 13% of the PDB patients, with two novel missense *SQSTM1* mutations identified, A381V and L413F. Two PDB patients carried a double *SQSTM1* mutation. The mean age of PDB onset was younger in the PDB patients with *SQSTM1* mutation (53.0 ± 10.5 years versus 60.5 ± 12.5 years, P= 0.05). PDB was more extensive in patients who carried a *SQSTM1* mutation with an increased mean number of affected bones (4.2 ± 3 versus 2.1 ±1.5, P=0.0002).
Conclusion: Mutations of *SQSTM1* are frequent in the French population. The presence of at least one *SQSTM1* is correlated with a younger age of onset and a more extensive PDB.

P0230. Comprehensive analysis of the *LRRK2* gene in sixty families with Parkinson's disease

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BACKGROUND: Mutations in the gene Leucine-Rich Repeat Kinase 2 (*LRRK2*) have been recently identified in families with Parkinson's disease. However, the prevalence and nature of *LRRK2* mutations, the polymorphism content of the gene, and the associated phenotypes remain poorly understood. OBJECTIVE: We performed a comprehensive study of this gene in a large sample of families with Parkinson's disease compatible with autosomal dominant inheritance (ADPD). METHODS: The full-length open reading frame and splice sites of the *LRRK2* gene (51 exons) were studied by genomic sequencing in 60 probands with ADPD (83% Italian). RESULTS: Pathogenic mutations were identified in six probands (10%): the heterozygous p.G2019S mutation in four (6.6%), and the heterozygous p.R1441C mutation in two (3.4%) probands. A further proband carried the heterozygous p.I1371V mutation, for which a pathogenic role could not be established with certainty. Thirteen novel disease-unrelated variants and three intronic changes of uncertain significance were also characterized. The phenotype associated with *LRRK2* pathogenic mutations is the one of typical PD, but with a broad range of onset ages (mean 55.2, range 38-68 years) and, in some cases, slow disease progression. CONCLUSION: On the basis of the comprehensive study in a large sample, we conclude that pathogenic *LRRK2* mutations are frequent in ADPD, and they cluster in the C-terminal half of the encoded protein. These data have implications both for understanding the molecular mechanisms of PD, and for directing the genetic screening in clinical practice.

P0231. The case of pyruvate dehydrogenase complex deficiency with antenatal beginning

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Premature girl was consulted by clinical geneticist on the 2nd hour of life due to the dysmorphic phenotype: palpebral fissures slant down, exophtalm, low-set dysmorphic ears, long philtrum, down-turned corners of the mouth, micrognatia, and rocker-bottom feet. The pregnancy history was complicated: on the 33rd week of pregnancy hydrocephaly, hypotrophy and oligohydramnion were diagnosed. Child was born prematurely on the 35th gestational week. At first she was interpreted clinically as a patient with possible chromosomal aberration or dysmorphic syndrome, but at the age of 4 days rapid disorientation occurred caused by increased lactic acid in serum (maximum10.6 mmol/l) and in cerebrospinal fluid (13 mmol/l). GCMS analysis of the urine showed markedly increased secretion of lactate and pyruvate. Serum pyruvate was also markedly increased with normal lactate/pyruvate ratio 3.5. MRI of the brain showed severe brain atrophy. The enzymatic studies for measurement the activity of pyruvate dehydrogenase complex (PDH) in the muscle showed significantly reduced activity. The enzymatic analysis from fibroblasts is still under investigation. The treatment according to the schedule PDH was initiated at the age of 6 days: thiamine 500 mg/die, biotin 1 mg/kg/die and coenzyme Q_{10} 10 mg/kg/die. Her diet is rich in fat and low in carbohydrates. This treatment lowered significantly the lactic acid level in serum and cerebrospinal fluid.
Conclusion: this was one of the first diagnosed cases of PDH deficiency in Estonia. Lessons to learn: investigating dysmorphic child, metabolic diseases should be kept in mind, specially in case of unexpected clinical course.

P0232. Peroxyzomal Disorders in Iran

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Peroxyzomes are responsible for a number of very important metabolic reactions, including synthesis of glycerol ethers, shortening VLCFAs (C24:O and C26:O), and oxidation of the side chain of cholesterol needed for bile acid production.
Peroxyzome biogenesis disorders (PBDs) are genetically and phenotypically related disorders that involve enzymatic activities of peroxyzomes. They are rare mostly autosomal recessive diseases characterized by multi-systemic structural and functional abnormalities. A number of biochemical abnormalities have been described in PBD patients including decreased levels of plasmalogen, and increased levels of VLCFA and cholestanoic acid derivatives. More than 25 different entities have been diagnosed in the last two decades. The diagnoses on suspected cases can now be confirmed precisely by detailed biochemical evaluation in some metabolic centers.

The most severe condition is the Zellweger syndrome, a condition due to the absence of functional peroxyzomes. Affected patients are severely ill, and show multiple congenital anomalies and neurological aberrations. There are specific biochemical tests for evaluating peroxyzomal functions. Accumulation of certain VLCFAs (C24:0, and C26:0); deficiency of plasmalogens, and elevation of phytanic acid are some of them.
Chondrodysplasia punctata is another example, they are genetically heterogeneous group of dysplasias having stippling of the epiphyses in infancy as a common feature. Peroxyzomal abnormalities only found in the rhizomelic type. A few cases of Zellweger syndrome and Chondrodysplasia punctata will be presented with a brief overview on biochemical background, clinical evaluation, and diagnosis.

P0233. Poland syndrome and paroxysmal supraventricular tachycardia: a sequence or just coincidental findings?

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Poland syndrome is a developmental disorder of as yet undetermined etiology. Several causes have been proposed for this syndrome, which include primary hypoplasia of the central brainstem nuclei, secondary brainstem nuclear degeneration, peripheral nerve involvement and mostly accepted sequence of prenatal subclavian artery disruption. Most cases are sporadic. The most consistent pattern of defects is unilateral hypoplasia or aplasia of the pectoralis major muscle and varying degrees of ipsilateral brachydactyly and syndactyly, less consistent features include hypoplasia of breast and/or nipple, other upper limb reduction defects, Sprengel anomaly, dextrocardia, rib and vertebrae anomalies. The syndrome is 3 times more common in males and 75% right-sided.
The presented patient was diagnosed with Poland syndrome at birth (there were aplasia of the left pectoral major muscle, brachydactyly and syndactyly of ipsilateral hand). Cardiac, abdomen ultrasonographic, intracranial duplex sonoscopic findings and chest X-ray were normal. The first paroxysm of supraventricular tachycardia was recorded at the age of 11 months during acute viral respiratory infection. More such episodes accurred during the 10 months period. Cardiac arrhythmias were treated with adenosine boluses, later amiodarone was added.
Conclusion: only one case of Poland-Moebius syndrome in association with cardiac arrhythmia was reported previously (Pierpont et al.). It is not known if this association is a sequence of the same etiology, or just the two coincidental factors.

P0234. A de novo paternal microdeletion of 6q, including SIM1, is not only associated with a Prader- Willi Like Phenotype (PWLP) but also with Basedow's disease and acanthosis nigricans

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Three different classes of 6q deletions with shared phenotypic features have been described (Hopkins et al. 1997). Rare cases with microdeletions indicate that within deletion group B (6q15q25) a distinct subgroup of patients with PWLP may result from hemizygosity of the small chromosome segment 6q16q21 including also the SIM1 gene (Faivre et al. 2005). We report about a 16-year-old girl with adipositas (BMI 30), severe mental retardation and other major features of PWLP. Basedow´s disease and acanthosis nigricans were seen in addition to macrocephalus, dental anomalies, uvula bifida and kyphosis. Cytogenetic and microsatellite analysis revealed a de novo paternal microdeletion including SIM1 at 6q16.3. Our study confirms the assumption that 6q-microdeletions including SIM1 are associated with obesity and PWLP. Recently it has been assumed that hemizyosity and/or functional loss of SIM1 is associated with hyperphagic obesity via a disturbed regulation of MC4R expression in the paraventricular nuclei of hypothalamus (Holden et al. 2005). Basedow´s disease and acanthosis nigricans have not been reported so far in the context of deletions including 6q16q21. These additional clinical features of our patient would indicate that hemizygosity for that 6q segment including SIM1 has additional neuroendocrinological effects, e.g. such as on the level of TRH and MSH.

P0235. Compound heterozygosity for mutations in *LMNA* causes a progeria syndrome without prelamin A accumulation

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LMNA-associated progeroid syndromes have been reported with both recessive and dominant inheritance. We report a two-year-old boy with an apparently typical Hutchinson-Gilford progeria syndrome (HGPS) due to compound heterozygous missense mutations in *LMNA* resulting in T528M and M540T. Both mutations affect a conserved region within the C-terminal globular domain of A-type lamins, shown to be affected in other progeria-like patients. The nuclei of our patient showed no prelamin A accumulation, nor did the dysmorphic nuclei reveal the lobulation typical for HGPS. Instead, nuclear blebs with reduced/absent expression of B-type lamins as well as honeycomb figures could be detected. The healthy heterozygous parents showed similar nuclear changes, though in a smaller percentage of nuclei. Treatment with a farnesyltransferase inhibitor to block the prelamin A processing resulted in accumulation of prelamin A and improved the nuclear phenotype. In conclusion, these findings suggest a critical role for the affected lamin A/C region in nuclear structure and support a major contribution of abnormal polymerisation to the progeroid phenotype. Prelamin A accumulation may not be the major determinant of the progeroid phenotype, in contrast to earlier suggestions.

P0236. A novel lamin A/C gene mutation in patient with progeroid phenotype

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Mutations in LMNA (lamin A/C) gene have been described in patients with several different clinical disorders, including Hutchinson-Gilford progeria syndrome (HGPS), mandibuloacral dysplasia (MAD1), Emery-Dreifuss muscular dystrophy (EDMD-2, EDMD-3), Charcot-Marie Tooth (CMT-2B1), Dunnigan-type familial partial lipodystrophy (FPLD), Limb-girdle muscular dystrophy (LGMD1B), atypical Werner's syndrome (aWRN), Seip syndrome and restrictive dermopathy (RD).
Progeria is an autosomal dominant condition which presents in early childhood with symptoms of premature aging and early death. Mandibuloacral dysplasia is an autosomal recessive disorder, characterized by mandibular hypoplasia, acroosteolysis, and progeroid facial appearance. These two diseases have been considered different clinical entities based on differences in phenotype and inheritance pattern.
We are describing a patient with failure to thrive, developmental delay, stroke, skin dyspigmentation, and facial dysmorphism. Brain magnetic resonance imaging with MR angiography revealed severe atheromatous disease and recruitment of collateral circulation. His history, physical exam and radiology findings were not completely consistent with either progeria or MAD which presented a diagnostic dilemma. Molecular studies were conducted and revealed a novel, heterozygous (412 G>A) mutation in exon 2 of the lamin A/C gene. Neither parent had a mutation.
HGPS and MAD are allelic and clinically overlapping conditions. Distinguishing between these diseases in a clinical setting has implications in predicting prognosis for the individual child and greatly influences genetic family counseling. Given the lack of clinical distinction in progeroid disorders, DNA testing for patients is a useful tool in evaluation.

P0237. ‚Progeroid syndromes' in genetic counseling practice - clinical characterization of 7 Belarusian patients

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Identification of disorder type among heterogeneous group of "progeroid syndromes" is important for genetic counseling due to similarity of main pattern of premature senility signs, but different inheritance influencing

for prognoses. Pathogenesis of pre-aging processes is still unknown, diagnosis is based on clinical findings and course.
We present 7 sporadic cases (5 females with normal intelligence; 2 males), examined due to "progeroid" appearance. Parents were young, healthy, non-consanguineous. Clinical diagnoses were "Wiedemann-Rautenstrauch syndrome" (girl aged 1.5 years showed neonatal progeroid symptoms, failure to thrive, lypodystrophy; boy 9.5 years old presented short stature, cachexia, borderline mentality, progressive senility features, hepatosplenomegalia, lymphohystiocytoses), "Werner syndrome" (girl 17 years old, female 40 years old), "unclassified" forms (3 patients): onset of senility signs was in childhood with progressing during follow-up examinations (8-18 years old). 2 girls presented similar picture: short stature, failure to thrive, hypotrichosis, "bird" face, protruding eyes, beaked nose, subcutaneous tissue loss, atrophic, scleroderma-like changes of skin, thin limbs, prominent joints, contractures, hyperkeratosis on soles, heart failure, large abdomen, hepatomegaly, diminished sexual development, diabetes mellitus (one girl), deafness (another one). Male showed progeroid appearance from 13 years old, joints contractures, severe regress of mentality. Laboratory data: chromosomal abnormalities, metabolic defects no found. Decreased replicative life-span of cultured skin fibroblasts was revealed in patient with Werner syndrome.
New cases of progeroid disorders must be collected for further delineation of phenotype and counseling improving. Differential diagnosis of 3 cases with unclassified "progeroid" condition (new "juvenil" form?) are discussed.

P0238. Molecular analysis of minisatellite sequence C_4GC_4GCG in CSTB gene in patients with progressive myoclonus epilepsy (EPM1).

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Progressive myoclonus epilepsy type 1 (EPM1; Unverricht - Lundborg disease) is an autosomal recessive disorder caused by mutations within the gene encoding cystatin B (*CSTB*). Clinical symptoms occur usually at age of 6 - 18 y. and embrace myoclonus, severe tonic-clonic epizodes, dementia, ataxia and dysarthria. The most common mutation is an expansion of a dodecamer repeat C_4GC_4GCG in 5" UTR of *CSTB* gene; the point mutations may occur as well. Normal alleles contain 2 or 3 copies of the repeat, whereas mutant alleles contain more than 30 repeats.
The polymorphism of C_4GC_4GCG in the Polish control group (300 alleles) and in the patients was analysed in this study.
DNA samples were obtained from leukocytes of peripheral blood from the patients and their family members. Analysis of C_4GC_4GC repeats in CSTB gene was performed by standard PCR reactions with specific primers labelled fluorescently and electrophoresis of PCR products in polyacrylamide gels.
The frequency of the 3-copy allele was 68% in the control group. In 5 of 9 referred patients all expanded alleles contained over 60 repeats. No case of the point mutations was found among our patients; all of them were homozygous. In 3 families the carrier status of both parents was confirmed. Molecular studies confirmed clinical diagnoses and occurrence of EPM1 in Poland.

P0239. Prune Belly Syndrome: a clinical study

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Prune Belly Syndrome is a rare disorder characterized by partial or complete absence of the abdominal muscles, cryptorchidism and urinary tract malformations. PBS syndrome occurs almost exclusively in males, with less than 3% occurring in female patients. We have studied 3 male patients aged 0-11 years with PBS in order to appreciate the frequency of different clinical features and to present some particularities. Physical examinations revealed deficiency of abdominal wall muscles and cryptorchidism in all patients. Investigations (renal ultrasound, CT, contrast voiding cystourethrogram, echocardiography) showed various abnormalities: hypoplastic right kidney in one patient, bilateral hydronephrosis in 2 patients, dilated ureters in all patients, large and thick-walled bladder in 2 patients, posterior urethral valve, vesicoureteral reflux and cardiac anomalies (ASD, PDA) in one patient. We have established the diagnosis of PBS based on the characteristic association of lack of abdominal muscles, cryptorchidism and urinary tract malformations. Differential diagnosis was done with other types of urinary tract anomalies (megacystis megaureter, urethral obstruction, primary vesicourethral reflux, neurogenic bladder). The plan for the management and the follow up of the patients will be presented. The prognosis depends on the degree of renal function compromise and the presence of extra urinary anomalies, especially cardiac. A possible case in a girl will be presented for discussion. In conclusion, we present a clinical study of PBS in order to illustrate this rare disorder, but also to discuss the importance of a complex medical specialist team for a correct diagnosis and management of the affected family.

P0240. Pseudoxanthoma Elasticum-like disorder with generalized cutis laxa and clotting deficiency represents a novel genetic entity

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A Caucasian female patient is presented who was diagnosed with Pseudoxanthoma Elasticum (PXE) because of peau d'orange skin lesions in the neck and limited angioid streaks in fundo. Skin biopsy showed fragmentation and calcification of elastic fibres. After puberty, she developed an increasing number of excessive, leathery skin folds, initially confined to flexural areas but gradually spreading towards the abdomen and limbs. Visual acuity remained normal. Decreased levels of the vitamin K-dependent coagulation factors were observed prior to surgery for a cerebral aneurysm. Three similar additional patients are currently being studied in a Belgian-French-Italian collaboration.
The present phenotype shows clinical overlap with PXE because of i) the initial skin manifestations mimicking the disorder, ii) the identical light microscopical findings and iii) the angioid streaks in fundo. However, several differences allow us to discriminate from PXE or cutis laxa. First, the skin involvement is more severe than in PXE, and not confined to the neck and flexural areas. Secondly, the retinopathy is mild and does not affect visual acuity. Thirdly, ultrastructural analysis showed mottled appearance of elastic fibres, with mineralization as small electron dense precipitates, not observed in PXE. Fourthly, analysis of the *ABCC6* gene, responsible for PXE, failed to reveal any mutation. Finally, the presence of elastic fibre mineralization and the retinopathy exclude known cutis laxa syndromes.
In conclusion, our data support the hypothesis that these four patients suffer from a new disorder, the molecular background of which is under investigation.

P0241. Complex rearrangements of chromosome 15 in two patients with Prader Willi syndrome

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Maternal UPD as a result of rescued trisomy increases with maternal age and accounts for 30% of cases of PWS. Two cases of maternal UPD in two unrelated children with mild clinical features of PWS are described. Methylation specific PCR showed one single band from the maternal allele, thus confirming the clinical diagnosis of PWS. FISH analysis with SNRPN probe in both patients revealed normal hybridization pattern. Microsatellite analysis in patient No 1 showed a combination of maternal iso and hetero-disomy of chromosome 15 while in patient No 2 the coexistence of heterodisomy or normal biallelic inheritance along with isodisomy is noted.
The coexistence of uniparental maternal iso- and heterodisomy could be explained by a recombination event before the 1st meiotic division followed by non-disjunction during the 2nd division and loss of the paternal homologue due to trisomy rescue.
The influence of complex rearrangements of chromosome 15 on the phenotype, when imprinted genes are included, is of great interest. Such cases are rare in the literature. The detection of a deletion by

FISH and abnormal methylation pattern could be sufficient for the confirmation of PWS diagnosis but provides no information for the exact molecular alteration leading to the disease. Microsatellite analysis of PWS/AS critical region and the telomere of the chromosome15 allows the detection of uncommon rearrangements.

P0242. PWS-like phenotype in a female affected with subtelomeric 15q26.3 deletion

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Objective: Deletions involving only the terminal region of the long arm of chromosome 15 are rarely reported. Commonly noted findings are prenatal-onset of growth failure, hypotonia and global developmental delay including speech impairment, dysmorphic features are only subtle. We report on a 7 year old female delivered at 35 weeks of gestation by cesarian section due to fetal bradycardia. She presented with proportionate dystrophic growth retardation (3rd percentile), delayed respiratory adaption, and hypotonia. No major malformation was stated but gavage feeding was necessary for several weeks. From the 2nd year of life she showed increasing appetite and later on foraging eating behaviour. Motor and particularly speech development remained poor. Aged 7 years she showed severe growth failure (-3.4 SD), and truncal obesity (BMI + 2.5 SD), hypotonia, friendly and happy disposition, fair complexion, microcephaly, broad forehead, hypertelorism, large mouth and rounded ears. A single palmar crease of the small digit was noted.
Lab investigations: Karyotyping and methylation testing for PWS/AS showed normal results. Molecular screening for subtelomeric rearrangements (MLPA) revealed a deletion on 15qter, which was confirmed by FISH. Parents' investigation was not possible so far.
Conclusions: Terminal deletion of 15q, and similarly ring chromosome 15, are known to be associated with growth failure, mental retardation and some malformations like diaphragmatic hernia. Prader-Willi like appearance and behaviour are reported here for the first time. Further investigations including refinement of the deletion size and screening for additional rearrangements which may contribute to this phenotype remain to be performed.

P0243. RAPADILINO syndrome in a patient without radial aplasia: the contribution of *RECQL4* gene mutation analysis

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We report the case of a 6-year-old boy who developed pre- and postnatal growth retardation, severe chronic diarrhoea and failure to thrive. Cheeks only presented a poïkilodermic appearance. Lack of eyebrows and eyelashes and absence of cataract were noted. Widespread café-au-lait spots were observed. Orthopaedic and radiologic investigations identified bilateral congenital radial head luxation, patellar hypoplasia but absence of thumb anomaly. This boy of normal intelligence is the first child from healthy unrelated Caucasian parents. Standard karyotype was 46,XY normal male, increased chromosome breakage anomaly screening was negative (Mitomycin-C). On molecular analysis of the *RECQL4* gene (8q24), a splice site substitution (IVS10-1 G>A) and a nucleotide substitution (L638P) in exon 7 were found. The mother carried the L638P substitution and the father was identified with the splice site mutation. These were so far unreported and not present in controls.
RAPADILINO eponym syndrome was suggested as a distinct entity due to the peculiar phenotypic features: Radial aplasia/hypoplasia-High Palate - Patellar Aplasia - Diarrhoea - LIttle size - Limb Anomaly-NOrmal Intelligence - Long NOse.
Recent molecular progress from *RECQL* gene family studies allowed delineating rather a continuum from Rothmund-Thomson to RAPADILINO syndromes.
This case emphasizes occurrence of mild limb involvement in a patient with cardinal features of RAPADILINO syndrome. Molecular analysis represents the most helpful approach and improves genetic counselling for this rare autosomal recessive disorder. Screening and follow-up recommendation for tumour risk - associated with *RECQL4* mutations in RAPADILINO syndrome - remain very uncomfortable.

P0244. A new mutation in *TP63* is responsible for early-aging features in Rapp-Hodgkin syndrome

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Increases in the number of allelic malformation syndromes have led to their classification according to their pathogenesis rather than their clinical specific phenotype. *TP63* mutations have been identified in several such syndromes characterised by autosomal dominant transmission and various combinations of ectodermal dysplasia, limb malformations and orofacial clefting.
We report on a family with four affected adult females presenting with Rapp-Hodgkin syndrome, an autosomal dominant clinical entity which associates anhidrotic ectodermal dysplasia with cleft lip and palate. Overlapping features with EEC syndrome (ectrodactyly, ectodermal dysplasia and cleft lip/palate) have led to the recent identification of mutations in the *TP63* gene, located on 3q27, in this condition. Our patients present typical clinical features of Rapp-Hodgkin syndrome, but also ophthalmic anomalies such as corneal dystrophy and early-aging features such as premature menopause (around 30 years). These latter findings have never been reported in this condition, and could be secondary to a new *TP63* deletion which has been identified in this family.

P0245. The influence of the polymorphic genes of the HLA antigenes class II on the human reproduction.

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The recurrent pregnancy loss (RPL) and unsuccessful attempt of in vitro fertilization (IVF) may have the same reasons and mechanisms. One of them may be sharing alleles in the HLA antigenes class II, which play significant role in maternal-fetal interaction immunology reaction at early stage of pregnancy. We have investigated 29 couples with RPL, 39 couples with IVF failure and 65 couples having at least one own child as a control group. The HLA genotyping has been done using the allele-specific nested amplification with specific primers. We have defined groups of alleles in gene DRB1- 12 groups, DQA1 - 8 and DQB1 - 12. The number of families having 1,2 and 3 shared groups of alleles has been counted. It has been revealed that the number of coincidence in three loci is 48.3% in RPL group, 43.6% in IVF group and 26.0% in control group (pRPL=0.044, OR=2.6(1.0-6.6) and pIVF=0.082, OR=2.2(0.9-5.1) accordingly). Also we have investigated distributions of separate alleles, genotypes and haplotypes and found the increased frequencies of DQB1 0302 and DQA1 0301 alleles among men and DQB1 0602 allele among women in RPL and IVF groups compared to controls.
In conclusion we can say that the presence of identical allelic groups on three loci gives twofold-increased risk of pathology of pregnancy. The risk of pregnancy pathology is considerably higher in presence of certain alleles, genotypes and haplotypes.

P0246. Mutations spectrum in X-linked retinitis pigmentosa in the Danish population

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X-linked Retinitis pigmentosa (xlRP) accounts for approximately 15 % of RP cases and represents the most severe subtype of this disease. Five distinct RP loci on the X chromosome have been proposed. The genes RPGR and RP2 have been identified accounting for 85-90% and 8-15% of xlRP cases, respectively. A mutational hotspot in ORF15 of RPGR was described.

We report the mutation spectrum of xlRP in the Danish population. Our study of 28 cases from 28 families with certain or probable xlRP represents all elucidated cases in the Danish population. RPGR and RP2 were screened by sequencing. We uncovered a mutation in 26 patients (detection rate 93%). 11 patients (42.3%) carried a mutation in the RP2 gene, in 8 patients (30.8%) the mutation was located in exons 1-14 of the RPGR gene and in 7 patients in ORF15 of the RPGR gene (26.9%). The most frequent mutation was p.S6del in RP2 which was found in 4 presumed unrelated patients. Additional genealogical studies, however, disclosed a common ancestry for two of them.
Twelve mutations were not reported previously. One splice site mutation and a larger deletion were further characterized. In one patient we found the integration of a 342 bp Alu element of the AluYb8 family into the coding region of ORF15, a rare form of mutation.
Conclusions: The proportion of RP2-mediated xlRP in the Danish population is higher than the proportion reported from other populations. Therefore strategies for diagnostic procedures must consider the mutation spectrum of the involved population.

P0247. Paternally inherited *MECP2* sequence variants in three girls with Rett syndrome

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More than 95% of girls with Rett syndrome (RTT) carry mutations in the *MECP2* gene on Xq28. Pathogenic sequence variants in this gene can also be detected in the majority of girls with *variant* RTT.
We report on the molecular analysis of *MECP2* in two girls with variant RTT and one with classical RTT. Patient 1 showed two novel nucleotide changes in the *transcriptional repression domain* (TRD) of *MECP2* (c.861C>T, p.A287A and c.881G>A, p.R294Q). Her healthy father and grandmother carried the same changes. Patient 2 showed a duplication of three nucleotides in exon 1 of *MECP2* (c.43_45dupGGA, p.G15dup). Further analysis revealed that the healthy father carried the same duplication. The third girl, who fulfilled the criteria for classical RTT, showed two sequence variants in exon 4: a duplication of 5 nucleotides in the TRD (c.750_754dupCCCCG, p.G252A*fs*X39) and a substitution of an arginine by a tryptophan at a non-conserved amino acid position at the C-terminal segment (c.1030C>T, p.R344W). While the first mutation (which is most likely disease causing) has not been described before, the latter was reported in a male displaying a “Rett-like” phenotype. Analysis of the family revealed that the frameshift mutation had arisen *de novo*, while the p.R344W mutation was inherited from the healthy father and the paternal grandmother. Our data show the difficulties in determining the pathogenic value of novel mutations in the *MECP2* gene, especially in girls with variant RTT. They also emphasize the importance of analyzing parental DNA to avoid incorrect interpretation of *MECP2* sequence variants.

P0248. Coexistence of Rett syndrome and Spinal Muscular atrophy (SMA) Type II. A case report

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Rett syndrome (RS) is an X-linked dominant neurodevelopmental disorder characterized by normal development until 6-12months of age, followed by regression with loss of acquired skills, gradual onset of microcephaly, stereotypic hand movements and psychomotor delay. RS is caused by mutations in *MECP2* (Xq28) or *CDKL5* (Xp22). SMA is a neuromuscular autosomal recessive disease characterized by progressive atrophy and paralysis of proximal muscles of the shoulder and pelvic girdle, caused by mutations in the *SMN 1* gene locus (5q11.2-13.2).
We report a 6-year-old girl who, at 2years of age, presented with centromelic weakness and psychomotor delay. The EMG showed diffuse neurogenic damage and the karyotype was 46, XX. SMA type II was diagnosed and molecular analysis (PCR-RFLP’s) revealed that both exons 7 and 8 of *SMN 1* as well as the exon 5 of the *NAIP* gene were deleted. At the age of 4years, there was onset of stereotypic hand-washing movements and epileptic seizures. Two years later, re-evaluation found severe psychomotor delay, microcephaly and hyperventilation/breath-holding attacks. An EEG showed background slowing with multiple spike-wave complexes in occipital leads. DNA analysis (DGGE and sequencing) identified the hotspot missense mutation R306C (c.916C>T) in exon 4 of the *MECP2* gene; subsequent analysis of the mother’s DNA sample was negative.
The combination of RS and SMA has not been previously reported. Thorough clinical evaluation that considered the coexistence of two rare monogenic syndromes in the same patient, in combination with DNA analysis, allowed accurate diagnosis, providing valuable information for the genetic counseling of the family.

P0249. Roberts syndrome: clinical spectrum, natural history and molecular study in two children of consanguineous Italian parents

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Roberts syndrome (RBS) is an Autosomal Recessive disorder, characterized by pre- and postnatal growth retardation, microcephaly, bilateral cleft lip and palate, tetraphocomelia and loss of cohesion at heterochromatic regions of centromeres and the distal portion of the long arm of the Y chromosome. The cytogenetic aspects are known as RS effect, Premature Centromere Separation, heterochromatin repulsion or “puffing” and “splitting”.
Recently, mutations in ESCO2 (establishment of cohesion 1 homolog 1) on chromosome 8p21.1 have been reported in RBS. ESCO2 mutations are also responsible for the form of SC phocomelia (pseudothalidomide syndrome) with heterochromatin repulsion (HS).We report the clinical, cytogenetic and molecular studies of a family with two children with RBS, a female and a male of the same sibship with consanguineous parents. A c1595delT mutation, in exon 10 of ESCO2 gene, was identified in both parents in a heterozygous condition The intrafamilial variability of the clinical spectrum and natural history represents an important aspect for the phenotype-genotype correlation in this very rare disorder.

P0250. Clinical, Orodental and Electron Microscopic Changes of Gingival Biopsy in Autosomal recessive Robinow Syndrome Suggest a Storage Disorder and a Midline Developmental Field Defect

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Robinow syndrome is a short stature syndrome with both autosomal dominant and recessive forms (OMIM 180700 and 268310) with some common Features to both forms. The present study aimed at correlating the orodental and clinical features as well as study of the electron microscopic changes of gingival specimens, for the first time in the medical literature, as an aid in the diagnosis and pathogenesis of the Robinow syndrome. The studied samples consisted of seven Egyptian cases, four males and three females, with ages ranging from 2 months up to 12 years. The clinical genetic evaluation of all cases included family history, pedigree analysis, clinical examination, detailed orodental examination, panoramic X-rays, as well electron microscopic study of gingival biopsies in two cases. All the cases were of the autosomal recessive form with positive parental consanguinity. Clinical examination revealed short stature, depressed broad nasal root, thick everted lips macrostomia, pseudo lower labial cleft, long philtrum, and hypertelorism with varying percentages. Skeletal malformations; were also identified. The orodental anomalies were hypertrophied gingiva, high arched palate, bifid tongue tip, mandibular hypoplasia and malocclusion. Two oral midline anomalies noted in this study; bifid tongue tip and pseudo lip cleft suggest that Robinow Syndrome is a midline field defect.
Histopathological pictures of electron microscopic examination of the gingival biopsies revealed destruction of intercellular bridges, widening

of intercellular spaces, presence of intercellular mocoid-like storage material, and intracellular vacuoles, suggesting metabolic storage, supported by the occurrence of hepatosplenomegaly in some cases.

P0251. Rothmund-Thomosn (RTS) Syndrome and Pulmonary Sarcoidosis: Case Report

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Rothmund-Thomson Syndrome(RTS) (OMIM# 268400) is a rare autosomal recessive disorder characterized by infantile poikilodermatous skin rash (depigmentation, hyperpigmentation, telangiectasia, and dermal atrophy), sparse hair or alopecia, abnormal teeth, dystrophic nails, juvenile cataracts, short stature, skeletal abnormalities, premature aging, hypogonadism, and predisposition to certain malignancies.

Here we describe a 17 and 10 year old Saudi Arab sisters with RTS. They both presented with the skin rash which appeared during infancy and progressed with time to the typical poikelodermatous lesions. Their course was complicated with bone marrow suppression and myelodysplastic syndrome. In addition, the oldest sister developed broncheactasis and bilateral axillary, prevascular, mediastinal as well as hilar lymphadenopathiies. The histopatological study of one of the enlarged mediastinal lymph nodes showed non-caseated granulomatous inflammation with features suggestive of sacroidosis. Serum angiotensin converting enzyme (ACE) level was significantly elevated which supported the diagnosis of sarcoidosis. Other etiological factors for granulomatous inflammation were ruled out. To our knowledge, this is the first report a patient with RTS and pulmonary sacroidosis.

P0252. Partial monosomy 7pter→p15 in a patient with Saethre-Chotzen syndrome

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Saethre-Chotzen syndrome represents one of the most common types of craniosynostosis inherited as an autosomal dominant disorder and most cases are sporadic. It is characterized by high penetrance and variable expressivity, leading to difficulties in clinical diagnosis. Some patients, who exhibit most of the diagnostic criteria of Saethre-Chotzen syndrome, have structural abnormalities of chromosome 7. The case of a 4 year old boy with developmental delay, notable dysmorphic features and a history of surgical repair of craniosynostosis is described. The craniofacial findings included premature fusion of cranial sutures, characteristic facial appearance and limb abnormalities. Based on the clinical examination of the patient and his parents, he was characterised as a sporadic case of Saethre-Chotzen syndrome. Conventional and molecular cytogenetic analysis of peripheral blood revealed partial monosomy of chromosomal region 7pter→p15 de novo. The TWIST gene, located on chromosome 7p21.1, is thought to be a negative transcriptional regulator involved in osteoblast differentiation and maturation and it is thought that haploinsufficiency of the gene can cause the disorder. The diagnosis of Saethre-Chotzen syndrome and the identification of the chromosomal abnormality in the patient facilitated genetic counseling of the family.

P0253. Partial monosomy 7pter→p15 in a patient with Saethre-Chotzen syndrome

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P0254. Spectrum and functional analyses of HSPG2 (perlecan) mutations in Schwartz-Jampel Syndrome : perlecan deficiency as a cause of severe myotonia

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Schwartz-Jampel syndrome (SJS) is an autosomal recessive condition with high clinical variability reported in the literature. Eight mutations in the *HSPG2* gene encoding perlecan, a heparane sulphate proteoglycan present within basement membranes, are described in 6 SJS families without a detailed clinical description. To assess the spectrum of mutations and the associated phenotype, we investigated one third of the reported SJS population.

Twenty-three unrelated families (35 patients) were screened for mutations in the *HSPG2* gene. Perlecan expression was evaluated in patients' cultured cells at the mRNA and protein level to examine the effect of the mutations. Retrospective clinical analysis was carried out for patients with perlecan mutations. Twenty-five perlecan mutations were identified among the thirty-five patients including genomic deletion, nonsense, frameshift, missense, and splice-site mutations. Their deleterious effect was confirmed by the reduced, though not abolished, secretion of perlecan by patients' cells. A distinct cellular pattern was observed between truncating and missense mutations with lower level of perlecan mRNAs for the former, and intracellular retention of perlecan protein for the latter. Retrospective clinical analyses of the cohort revealed that permanent muscle stiffness, mostly apparent in the face, was the hallmark of the disorder. By contrast, chondrodysplasia was more variable, and was absent in two cases.

Schwartz-Jampel syndrome may be defined as a progressive autosomal recessive disorder with early childhood onset that associates mask-like face, neuromyotonia, and variable osteochondrodysplasia, with deficiency in perlecan secretion. Two cellular mechanisms, nonsense-mediated mRNA decay, and protein quality control pathway, are involved in this deficiency.

P0255. Ion channel mutations in inheritable idiopathic epilepsy

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Epidemiological research has provided evidence that in about 40% of patients with epilepsy, a genetic predisposition underlies the idiopathic disorder. Over the past decade, a number of genes have been identified that associate with rare monogenic forms of idiopathic epilepsy. Most of the genes encode for voltage- or ligand-gated ion channels or components thereof. For example, the SCN1A gene encodes for the brain voltage-gated sodium channel α_1 subunit ($Na_v1.1$). Mutations in the SCN1A gene have been found in substantial percentage of severe myoclonic epilepsy of infancy (SMEI or Dravet syndrome), borderline SMEI (SMEB), intractable childhood epilepsy with generalized tonic-clonic seizures (ICEGTC) and generalized epilepsy with febrile seizures

plus (GEFS+). Since a year the Department of Medical Genetics Utrecht offers DNA-diagnostic testing for the SCN1A ion-channel gene. SCN1A mutation analysis is performed on more than 100 probands. A start of a profound phenotype-genotype correlation was made for the first 47 probands. In 16 of 47 (34%) probands a mutation was found. The yield was highest in SMEI and SMEB patients, intermediate for GEFS+ and lowest for other (intractable) epilepsies. Mutations were localized throughout the protein, however, consistent with literature data, a clustering was found in the loops between segments 5 and 6. We will describe genotype-phenotype correlations and present our approach to functionally characterize the detected mutations.

P0256. X-linked nonsyndromic 46,XY sex reversal due to a cryptic Xp21 duplication including DAX1.

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Xp21 duplications including the DSS locus can cause sex reversal in males, usually in association with mental retardation and physical anomalies. A nonsyndromic 46,XY sex reversal with normal *SRY* was diagnosed in a 14-year-old girl (patient 1) and in her 27-year-old maternal aunt (patient 2). They both presented with complete sex reversal, including normal vagina and cervix, hypoplastic uterus and fallopian tubes. No gonads were identified by sonography in patient 1. Streak gonads were detected in patient 2 and they were surgically removed. On histological examination, primordial sex cords with absent germ cells and a very few Leydig cells were observed. Physical anomalies were absent, measures were within normal limits, intelligence was normal. Genetic analyses were carried out by FISH on interphase nuclei first, and then by semi-quantitative PCR. Probe RP11-662D2, containing *DAX1,* appeared to be duplicated by FISH in both 46,XY female individuals. Three copies were detected in the obligate carrier mother of patient 1, and two copies in her 46, XX healthy sister. Adjacent probes RP11-377J16 and RP11-540E20, lying proximally and distally to RP11-662D2, at a distance of about 1 Mb from each other, were not included in the duplication interval. *DAX1* copy number was evaluated by semi-quantitative PCR, and results were fully consistent with FISH data in a total of four repeated experiments. To the best of our knowledge, this is the first report of a nonsyndromic familial X-linked 46,XY sex reversal due to *DAX1* duplication.

P0257. A New Syndrome? A case report with short broad terminal phalanges

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The proposita, a 7 years old boy, was the second born to related parents. His physical findings was included short stature, moderate microcephaly, short broad terminal phalanges, broad toe and thumbs, short-broad nails with inability to flex the knees. He had down slanting palpebral fissures, thin lips, micro-retrognathia and long-smooth philtrum. His trunk was narrow and he had pectus carinatum.
His X-rays findings revealed impressio digitalis of calvarium, end plate irregularities of vertebral bodies and epiphyseal irregularities of knees. Especially, terminal phalanges of feet and hands were very short.
Shortness of terminal phalanges and flexion deformity of knee were major signs of the proposita. These findings were suggesting a new syndrome

P0258. A case of short stature with motor mental retardation, congenital cardiac anomalies, agenesis of uterus and ovaries and unusual craniofacial dysmorphology

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Short stature is defined as a condition in which the height is equal to or greater than 2 standard deviations (SD) below the mean height for a given age and sex. Pathological short stature can be distinguished from normal variants by careful evaluation of the patient by means of analyzing the growth parameters. More than 600 genetic syndromes have been reported in patients with short stature; most commonly achondroplasia, Turner Syndrome and Down Syndrome.
Here we report a 13 year-old girl who was the first child of non-consanguineous parents. She was of short stature accompanied with motor and mental retardation and recurrent respiratory and genitourinary infections. She had a coarse face with a broad forehead, puffy eyes, beaked nose and low-set ears. She also had a short neck, widely spaced nipples and pectus excavatum. Her cardiologic examination revealed atrial septal defect, mitral valve prolapsus and atrial septal aneurysm. Abdominal ultrasonography showed agenesis of uterus and ovaries; brain scans revealed dilatation of the third and lateral ventricles. Growth hormone deficiency was observed during the evaluation of GH/IGF-I axis. All other laboratory tests including metabolic screening and karyotype were noted to be unremarkable. In conclusion, the peculiarity of this case is that it may be a new clinical dysmorphological syndrome which has not been previously described and which may contribute to the understanding of the genetic basis of short stature.

P0259. Autistic Regression in a child with Russell - Silver Syndrome and UPD of Chromosome 7, a rare clinical presentation

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Silver-Russell syndrome (SRS) is a heterogeneous syndrome which is characterized by severe intrauterine and postnatal growth retardation and typical dysmorphic features. In 7-10% of SRS patients, a maternal uniparental disomy of chromosome 7 (UPD7) can be detected.
We describe a 4.5 y old boy, with SRS and UPD of chromosome 7. Along with the clinical findings that are described in this syndrome he had sever developmental delay which is not commonly found in these patients and an autistic regression that was not described before in this syndrome.
A possible explanation for the autistic regression might be the tendency for undetected hypoglycemic events in children with SRS. Another explanation is that the autism is not a part of SRS but is due to the chromosomal abnormality. A rare but important type of abnormality that accounts for some of cases of autism is uniparental disomy (UPD). There is a well established association of UPD with autism and the imprinted 15q11-q13 Prader-Willi/ Angelman syndrome (PW/AS) region. Recently there was a case with UPD of chromosome 1 and autism.
This case suggests an association of autism with a locus on chromosome 7. UPD can cause disease by two mechanisms. First, imprinted genes are subject to selective expression that depends on the parental origin. Second, UPD can also cause disease by unmasking recessive mutations that fall within regions of isodisomy.

P0260. Clinical and radiographic findings in eleven patients with metatropic dysplasia, demonstrating longterm natural history.

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We present clinical and radiographic data from eleven cases of metatropic dysplasia (MD), ranging in age from 20 weeks of gestation to 70 years, with 30-year follow-up in three cases. Included in this cohort is a pair of sibs (aged 56 and 52 years) and a father and daughter (aged 70 and 33 years).
Two patients died in infancy (aged 3 months and 4 months) from laryngeal/upper respiratory tract dysfunction while a third infant had a respiratory arrest secondary to severe laryngotracheomalacia. Longterm intellectual function is normal in the five individuals surviving to adulthood. Progressive and severe kyphoscoliosis occurred in all three patients followed over 30 years, despite having several spinal surgical procedures. Overall functioning with regard to activities of daily living remains reasonable in all five adult patients (ranging in age from 33 to 70 years) and joint and back pain were not reported as being significant in these patients. Final adult height ranged from 110-135cms.

The current classification of metatropic dysplasia into various subtypes, based on radiographic findings, is unclear. We suggest that this condition might be caused by the pleiotropic effects of a single dominant gene, with gonadal mosaicism reconciling sib recurrences.

P0261. X inactivation skewing and recurrent spontaneous abortions in a Greek population

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Recurrent spontaneous abortion (RSA) affects (1-2)% of couples. In up to 50% of the cases no etiology is identified (idiopathic RSA). Normally, during embryogenesis one of the two X chromosomes in female mammals is inactivated randomly. Skewed X-chromosome inactivation (XCI) is the preferential inactivation of the one (maternal or paternal) allele. Skewed XCI has been correlated with "idiopathic" RSA. We have studied 73 Greek women with idiopathic RSA and 79 controls for their X-chromosome inactivation pattern. Control women have two or more succesfull pregnancies.

Method: Selection of informative women (heterozigosity of HUMARA locus). Methylation sensitive assay (HpaII digestion).

Results: The ratio of heterozygotes was 72.6% (53/73) in women with RSA and 70.9% (56/79) in control group.

Of the 53 informative women with RSA, 5 (9.4%) showed extreme skewed XCI (> 90%) and 2 (3.8%) moderate skewed XCI (between 70% and 90%). Of the 56 heterozygous controls, 2 (3.6%) showed extreme skewed XCI and 4 (7.1%) moderate skewed X inactivation.

Statistical analysis: Frequency of extreme skewed X-inactivation between RSA women and controls was not significantly different (p=0.262). Similarly, no difference was found in the rate of any other degree of skewed X-inactivation between the two groups (p=0.772).

Conclusion: There is no association between idiopathic RSA and skewed XCI.

P0262. Two multiplex test systems for SMA therapy control

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Spinal muscular atrophy (SMA) is a frequent autosomal recessive disease characterized by degeneration of spinal cord anterior horn cells and muscular atrophy. Two highly homologous SMN genes are mapped on chromosome 5q13: SMN1 (telomeric copy, SMN^T) and SMN2 (centromeric copy, SMN^C). The disease is caused by mutation or deletion in SMN1 gene, which differs from the SMN2 gene only in five nucleotides. One of these differences is located in the exon 7 splicing enhancer that results in an incorrectly spliced transcript lacking exon 7 (SMNΔ7) for the SMN2 gene. More than 94% SMA patients have homozygous deletions in SMN1 exon 7, but not in the SMN2 gene. It is known that SMN2 expresses about 10% of full-length mRNA and this form may influence the clinical presentation of the disease. Recent reports about therapies for SMA contain approaches to increase the expression of full-length SMN2 gene transcript from by treatment of phenylbutyrate or valproic acid.

We have developed two multiplex test systems for quantitative estimation of SMN full-length mRNA and SMNΔ7-forms in cell culture and blood samples. The first one consists of specific primers for SMNΔ7 and SMN full-length with conservative TaqMan probes. The next system includes the universal SMN-primers and specific TaqMan probes for splice SMN mRNA forms. The level of both SMN- types mRNA compare with level of GAPDH gene expression. Cultured skin fibroblasts from SMA type II and SMA type III patients are used for dosage of valproic acid and for further evaluation of the developed multiplex test systems.

P0263. Ischiopatellar or small patella syndrome is secondary to mutations in the TBX4 gene: confirmation in a large family and clinical description

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Small patella syndrome (OMIM 147891) is a rare autosomal-dominant skeletal dysplasia characterised by patellar aplasia or hypoplasia, anomalies of the pelvis and feet and also sometimes discrete micrognathia and cleft or high arched palate. Mutations in TBX4 have been identified in 5 families and one isolated case so far. We report on a large family presenting with this condition, in which we have identified a frameshift mutation in TBX4. Clinical aspects and x-rays allowed us to discuss the different features of this condition, such as disrupted ossification of the ischia and inferior pubic rami in infancy, abnormal upper femora, infra-acetabular axe cut, elongated femoral necks, flattened and widened proximal femoral epiphyses, hypoplasia of the lesser trochanter, tarsal anomalies, and absent or hypoplastic patella responsible for knee instability later in life. In this family, several individuals also presented with non previously reported features such as dental anomalies and delayed puberty in affected women. TBX4 encodes a transcription factor with a strongly conserved DNA-binding T-box domain that is known to play a crucial role in lower limb development in chickens and mice (TBX4 is not expressed in the upper limbs). However, TBX4 is not sufficient to determine limb-specific morphologies and is interacting with several other genes. TBX4 expression has also been identified in the lungs and in the gut during embryo development, but nothing has been reported on dental expression so far. We discuss the clinical aspects of this condition, and the molecular mechanisms involved in limb morphogenesis.

P0264. Evidence that multiple genes in 17p11.2 contribute to the clinical spectrum of Smith-Magenis syndrome

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Smith-Magenis syndrome (SMS) is a complex disorder characterized by speech and motor delays, craniofacial anomalies, and significant neurobehavioral problems, including self-injury, sleep disturbance, aggression, and explosive temper tantrums. SMS is caused by mutation or deletion of the retinoic acid induced 1 (*RAI1)* gene on chromosome 17p11.2. In order to analyze the role of genes in the 17p11.2 region in this disorder, we evaluated the molecular and clinical findings in 32 SMS patients carrying either a 17p11.2 deletion or a mutation in the *RAI1* gene. Using FISH, we determined that 8/32 individuals carry a common ~3.5 Mb deletion encompassing the 17p11.2 region from *TNFRSF13B* to *ULK2*. Smaller deletions were found in 9/32, while 4 individuals carried larger and/or atypical 17p11.2 deletions. *RAI1* mutations, including single base and large intragenic deletions and missense mutations, were identified in 11/32 patients. Genotype:phenotype analysis was performed by comparing the features of patients with deletions and to those with mutations in *RAI1* using Fisher's exact test. Phenotypic comparisons show that cardiac anomalies, speech and motor delay, hypotonia, short stature and hearing loss are associated with 17p11.2 deletions rather than with *RAI1* mutations at statistically significant proportions (p <0.05). Interestingly, data show that patients with mutations in *RAI1* are overgrown, with height and weight >97thcentile. Further, patients with smaller deletions show features similar to those with *RAI1* mutations. We conclude that while *RAI1* is the primary gene responsible for the core features of SMS, other genes within 17p11.2 contribute to the variability and severity of the syndrome**.**

P0265. A P170R heterozygous mutation in the *SOX9* gene in a girl with subtle radiological features of campomelic dysplasia

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Campomelic dysplasia (CMD1, OMIM#114290) is an autosomal dominant syndrome characterized by bowing of the lower limbs, minor

facial anomalies and respiratory distress. Sometimes sex reversal can be seen. CMD1 is caused by mutations in the *SOX9* gene.
We present a 14-month-old girl with a P170R heterozygous mutation in the *SOX9* gene. She is the second child of non-related parents. She has a dolichocephalic skull, high arched palate, micrognathia, flat nasal bridge, low set ears and laryngotracheomalacia for which a tracheostomy was performed. Skeletal survey showed 11 pairs of ribs, delayed ossification of distal femoral epiphyses and brachymesophalangy II and V. The X-rays were reviewed by the European Skeletal Dysplasia Network (ESDN). Subtle radiological features of CMD1 were noticed. Most reviewers felt that the scapulae were slightly small, the thoracic pedicles were poorly delineated and hypoplastic and that there was a slight bowing of the femurs. Previously a patient with the same mutation and classical signs of CMD1,
including Pierre Robin sequence, low-set ears, bowing of tibiae and hypoplastic scapulae, was described. This patient died 4 weeks after birth (J.Meyer et al; 1997). These two cases illustrate that genotype/phenotype correlations are (almost) impossible in CMD1.

P0266. Surfactant protein B polymorphism in infants with lung failure from Russia

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Respiratory diseases are among the most common causes of death worldwide. Pulmonary surfactant is a mixture of lipids and proteins, which is essential for normal lung function. Alterations in surfactant composition have been reported in several lung diseases. It is known also, surfactant protein B (*SP-B*) gene is polymorphic.
To investigate the possibility of variable genetic susceptibility to RDS and pneumonia in neonates from Russia, we examined the association between RDS, pneumonia and a polymorphism in intron 4 of the *SP-B* gene.
We analyzed genomic DNA of 108 infants with respiratory distress-syndrome (RDS) and/or congenital pneumonia and 104 healthy term neonates from Russia by means of polymerase chain reaction.
On the basis of χ^2 analysis, the SP-B intron 4 alleles distribution did not differ between ill and healthy patients (p=0,19) (Table 1). There were only 0,9% infants with the invariant /deletion genotype in analyzed group and 8,7% in control group (p=0,02, χ^2=5,43). We did not observe this genotype among the neonates with RDS (p=0,03, χ^2=4,83).
We suppose the invariant /deletion genotype is protective for such congenital lung disease of neonates from Russia like RDS and pneumonia. So our results is not similar to those reported earlier. It is possible due to ethnic differences of Russian population.

Table 1. Distribution of the SP-B intron 4 alleles.

Alleles	All patients(N=216)	Control(N=208)
Invariant	205 (94,4%)	192 (92,3%)
Deletion	3 (1,4%)	9 (4,3%)
Insertion	8 (3,7%)	7 (3,4%)

P0267. Spondylo-epiphyseal dysplasia with primitive carpometacarpal osteolysis: a new dominant osteodysplasia

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Spondylo-epiphyseal dysplasia (SED) is a clinically heterogeneous condition encompassing many skeletal disorders, affecting the spine and the proximal epiphyses of the long bones. We report a new dominant form of SED observed in a woman and her son. The main features are 1) SED with X rays reminiscent of X-linked spondylo-epiphyseal dysplasia tarda (a form of SED that has no manifestation in females) leading to severe scoliosis in late childhood,present in a women and 2) primitive, progressive osteolysis of carpal bones, metacarpals and phalanges that developed in adulthood. Our family appears to be the first observation of an association of SED and acro-osteolysis.

P0268. *Homeobox gene PAX9 variation in a family with Hypohidrotic Ectodermal Dysplasia.*

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The term Ectodermal Dysplasia (ED) covers a heterogenous group of conditions affecting ectodermal organs including hair, teeth, nails, and glands. The term Ectodermal Dysplasia covers a heterogenous group of conditions affecting ectodermal organs including hair, teeth, nails, and glands. Hypohidrotic ectodermal dysplasia, EDA, the most common and best-known ED, is usually inherited as an X-linked semidominant trait, although rarer autosomal dominant and recessive forms exist. Affected males show severe oligodontia or anodontia, and abnormalities in tooth shapes. Anomalies are seen in both primary and permanent dentitions.
Further analysis established the causative mutation as a sub-microscopic deletion encompassing the PAX9 gene, thus, suggesting that haploinsufficiency of PAX9 leads to tooth agenesis. Here we report the characterization of a small nuclear family. Mandibular incisors, premolars, molars, and maxillary incisors, canines, premolars, molars are the teeth most often missing or having conical crowns in the some of current family members. Permanent teeth showed reduced mesiodistal dimensions and shorter root lengths than normal teeth. Primary teeth were normal in size, shape, and number. The single-strand conformatioanal polymorphism (SSCP) was used enabled the detection of a possible mutation in exon 2 of PAX9 in our family. Current results report variable patterns of homeobox gene in some members who has distinct findings of dental abnormalities in HED syndrome.

P0269. Huntington's disease genetic testing standards

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Huntington's Disease (HD) is a neurodegenerative disease that affects 4 to 7 individuals per 100,000. HD is an autosomal dominant inherited disease that is associated with an expansion of a trinucleotide (CAG) repeat located on chromosome 4. Individuals with 26 triplet repeats or less are normal, and while those with 27 to 35 repeats may not show symptoms themselves, their future generations may have the disease. Individuals with 36 to 39 repeats are at risk, while 99% of HD patients have 40 or more CAG triplet repeats. International cooperation with the Eurogentest community as well as NIST and the CDC is needed to promote a strategy to initiate the acceptance and recognition of validated materials to serve as international standards for genetic testing. In response to the need of standards for Huntington's Disease genetic testing, NIST, is validating a panel of Huntington's Disease genomic material for use as standard reference materials (SRM) for HD testing. The panel of standards (each consisting of two alleles) include a pair of homozygous alleles, closely-spaced normal alleles, normal/expanded alleles, borderline/expanded alleles, closely-spaced expanded alleles, and normal/large expanded alleles. Future coordinated efforts between NIST, ACMG, CDC, and the Eurogentest community will increase the number of standards for genetic testing such that the health care community has the positive and negative controls needed to significantly impact diagnostic accuracy especially in difficult and ambiguous clinical situations.

P0270. Autosomal recessively inherited Stickler syndrome

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Hereditary hearing loss is a very heterogeneous condition for which at the moment more than 70 loci and several genes are identified. Of these genes Connexin 26 (GJB2) is responsible for more than half of the recessively inherited cases of pre-lingual deafness. In about ¼ of cases additional abnormalities exist and the deafness is part of a syndrome.
We describe a consanguineous Moraccan family with four children affected by congenital hearing loss. Recessive inheritance was probable, but no mutation could be shown in Cx 26 or in the PDS gene.
Years later additional symptoms in the form of skeletal and ocular problems lead to the diagnosis of Stickler syndrome. DNA analysis

showed a homozygous mutation in the COL9A1 gene in the four patients, and a heterozygous mutation in both parents, fitting the recessive nature of the disorder. This family is the first case of recessively inherited Stickler syndrome. Uptill now 3 autosomal dominant forms of Stickler syndrome have been described, caused by mutations in COL2A1, COL11A1 and COL11A2. These forms differ in severity of the deafness and in the presence of ocular manifestations. Our family showes a different kind of vitreous changes, less pronounced facial features, and short stature.

P0271. Mosaic subtelomeric deletion of chromosome 10q in a girl with psychomotor retardation and dysmorphism.

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We report on a 27-month old girl with feeding difficulties, hypotonia, psychomotor delay, strabismus, and a facial dysmorphism. Family history was unremarkable. Brain magnetic resonance revealed no abnormalities. Standard karyotype was normal 46,XX, but analyses for subtelomeric rearrangements using MLPA (Multiplex Ligation dependent Probe Amplification) on blood showed a slightly diminished intensity for the telomere 10qter probe. FISH analysis confirmed the presence of a deletion of the subtelomeric region 10q present in approximately 35% of the analysed mitoses and interphase nuclei. FISH analyses on buccal cells confirmed the presence of a subtelomeric 10q deletion in approximately 44% of the cells. Subsequent FISH analyses with specific probes are ongoing in order to define the exact localization of the breakpoint.
Only a few cases with pure subterminal 10q deletion have been reported so far. The clinical findings in these patients consist mainly of developmental delay, short stature, behavioural anomalies, strabismus, and dysmorphism. The clinical findings in our patient are concordant with those of a subtelomeric 10q deletion. A mosaic subtelomeric 10q deletion has, to our knowledge, not yet been reported so far.
We would like to stress the importance of the possibility of the presence of a subtelomeric deletion in a mosaic form, detectable by MLPA and confirmed by FISH analyses.

P0272. Monosomy 7qtel and trisomy 19qtel in two unrelated patients

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Subtelomeric rearrangements are relatively common abnormalities among severely mentally retarded patients, particularly if the latter is associated with dysmorphism, congenital malformations, growth defects or a family history of mental retardation. The wide variety of subtelomeric chromosome abnormalities is in frame with the diversity of phenotypes among positive cases. Nevertheless, as the number of patients reported increases, recognisable patterns emerge pointing at particular chromosomal defects.
We have been using fluorescence in situ hibridisation (FISH) with subtelomeric probes to detect cryptic rearrangements in patients with mental retardation and a “chromosomal phenotype”, whose conventional karyotypes are normal.
We present two unrelated patients who were independently found to have monosomy of 7qtel and trisomy of 19qtel [der(7)t(7;19)(qtel;qtel)pat]. They are both severely mentally retarded and hypotonic. They have poor growth of prenatal onset, microcephaly, a round face with broad forehead, broad nasal bridge, short nose with anteverted nares, short wide philtrum, downturned corners of the mouth and thin lips. Both have imagiological abnormalities of the frontal lobes, and minor congenital heart defects.
Complex rearrangements make it difficult to ascertain which individual chromosomal defect is responsible for each phenotypic characteristic. Monosomy 7qtel is however noteworthy, in that two important genes have been ascribed to this region: the homeobox HLXB9, for dominantly inherited sacral agenesis, and Sonic hedgehog, the major gene causing holoprosencephaly. Interestingly, neither patient has sacral defects or anorectal anomalies, nor do they exhibit clinical features or CNS malformations suggestive of holoprosencephaly, corroborating the notion that the holoprosencephaly spectrum with 7qter deletion is extremely variable.

P0273. Autosomal Dominant Familial Thoracic Aortic Aneurysm, (TAAD2) associated with a heterozygous TGFBR2 gene mutation.

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Transforming growth factor beta type II receptor (TGFBR2) gene has been recently reported as associated with Familial Thoracic Aortic Aneurysm, designated TAAD2 (MIM%608967). Two further syndromes are allelic at the same locus: Marfan Syndrome type 2 (MFS2, MIM#154705) and Loeys-Dietz Syndrome (LDS, MIM#609192). The three conditions share the aortic dilation/aneurysm trait. We here describe a novel TGFBR2 gene mutation in a four-generation family with autosomal dominant aortic dissection. Mutational analysis was performed on genomic DNA. The TGFBR2 gene was amplified and sequenced using intron-specific primers for amplicons containing exons. Controls were 204 chromosomes from unrelated healthy controls. Linkage was assessed with two FAM-labeled microsatellites: D3S2336 and D3S1293. The W405R mutation (exon 6) of the TGFBR2 gene was identified in the proband, a 49-year old female with type B aortic dissection at the age of 48 years. Furthermore, the mutation was also found in her sister (who suffered acute aortic dissection), and in the youngest brother who presented a type A aortic dissection. Two additional sibs are unaffected and non-carriers. The three affected sibs showed identical D3S2336 and D3S1293 make-up. In TAAD2, the first diagnosis in the family is usually done after an acute, potentially fatal event. The normal skeletal and ocular phenotypes of these patients raise the problem on how to diagnose them before the occurrence of an acute dissection. A further problem is how to monitor and protect carriers because the penetrance is complete by the adult age, and the aortic size at dissection seems lower that five cm.

P0274. Diagnosis of HbE by an allelic discrimination analysis.

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Beta-thalassemia/ Hb E is one of the most common form of thalassemia in Thailand. These patients display a wide range of clinical severities, from nearly asymptomatic to transfusion-dependent thalassemia major. Their Hb F levels are variable and overlap with those of homozygous Hb E subjects. Differential diagnosis of these hemoglobinopathies is therefore problematic. In this study, an allelic discrimination analysis of Hb E was developed based on the multiplexed and end-point PCR . The presence of two primer/probe pairs in each reaction allows genotyping of Hb E at the single-nucleic polymorphism (SNP) site. The method was validated on 90 subjects including 16 Hb E heterozygotes, 30 Hb E homozygotes, 14 beta-thalassemia/ Hb E and 30 normal subjects. The results of Hb E diagnosis by SNP analysis were 100% in accordance with the results obtained by DNA sequencing technique. In conclusion, this approach should prove useful in differential diagnosis of Hb E in populations with a high frequency of Hb E and beta-thalassemia/ Hb E.

P0275. Co-inheritance of IVS II-1(G>A) beta globin gene mutation with a Delta-chain mutation [Delta116 Arg>Cys(G18) (CGC>TGC)], HbA_2-Troodos, in an Iranian family.

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The **β**-thalassemia (thal) minor phenotypes with normal Hb A_2 levels and decreased MCV and MCH values are relatively rare **β**-thal traits. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) revealed the IVS-II-1 (G>A) mutation in the **β**-globin

gene of the proband and her father. Direct sequencing of the **δ**-globin gene of the proband and her father also revealed a previously reported variant called Hb A_2-Troodos [**Delta** 116(G18)Arg**>**Cys] (in *cis*) with the **β**-globin gene mutation. This is the first case report of Hb A_2-Troodos in association with the β^0 IVS-II-1 (G**>**A) mutation. Reduced Hb A_2 expression by a concomitant Hb A_2 **β**-thal in *cis* or *trans*, may cause problems in carrier diagnostics, and eventually in genetic counseling and prenatal diagnosis when insufficient molecular analyses are performed.

P0276. Genetic and environmental risk factors in perinatal arterial stroke: Data from a cohort of 56 mother/child pairs

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Perinatal arterial stroke (PAS) is the most common cause of hemiplegic cerebral palsy, and thrombophilic factors appear critical in its causation. We evaluated demographic, historical and prothrombotic risk factors in 56 patients with PAS and their mothers between 1997 and 2005. PAS was confirmed by cranial imaging (CT/MRI). Boys predominated 33:23. There were 3 twin sets. Ethnicity was 31 Hispanic, 20 Caucasian and 5 other. Most were term (57%) and 18% were post dates. Three were growth restricted whereas 10 were large for gestational age. 25% of mothers reported decreased fetal activity; 9 (16%) had preeclampsia; 26 (30%) had emergency Cesarean section. Eight placental exams revealed 7 with abnormalities. 66% of children presented with seizures whereas 34% had a later presentation with cerebral palsy.
Prothrombotic risk factors evaluated were Protein C, S and Antithrombin III activities, Leiden Factor V, Prothrombin 20210, MTHFR C677T and A1298C, Lipoprotein (a), homocysteine and anticardiolipin antibodies. Abnormalities were found in 27/49 mothers (55%) and 30/55 (55%) children. Thirty-nine of the pairs (70%) had at least one abnormality in mother, child or both. Eight children and 10 mothers had more than one prothrombotic factor. Four children had patterns of birth defects or syndromes. Long term sequelae included cerebral palsy (90%), microcephaly (74%), cognitive impairment (67%), and seizures (42%). Three children died ; only 2/53 surviving children are normal.
PAS is the result of multifactorial, synergistic fetal and maternal factors among which genetic prothrombotic factors, both fetal and maternal, appear pivotal.

P0277. A case with Toriello-Carey syndrome

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Toriello-Carey syndrome (MIM #217980) is a rare multiple malformation syndrome characterized by agenesis of corpus callosum, telecanthus, short palpebral fissures, small nose with anteverted nares, Robin sequence, abnormal ears, redundant neck skin, laryngeal anomalies, cardiac defect, short hands, and hypotonia. There is also a considerable overlap in cardinal symptoms between Ohdo (MIM #249620) and Toriello-Carey syndrome suggested that these two conditions could be allelic. The only distinction between Toriello-Carey and Ohdo syndromes is rare associations seen in Toriello-Carey but not in Ohdo syndrome.
Here, we present a female case manifesting both typical and less common findings, such as respiratory distress, hoarse voice, hearing loss, abnormal rib number, simian-line, anterior placed anus, triangular open mouth, high palate and sacral appandage, of the syndrome. Triangular open mouth, hoarse voice and anterior placed anus were only described ones and never confirmed before. Presence of these defects in our case support that, they are a part of the phenotypic spectrum of Toriello-Carey syndrome. Additionally, our case has pericallosal lipoma which is an unhiterto described finding.
To the best of our knowledge, this is the first Turkish patient with typical severe phenotype of Toriello-Carey syndrome including early infancy death. It is previously suggested that males are more severely affected than females in Toriello-Carey syndrome. Evaluation of previously reported well described 21 cases indicate that there is no significant sex difference in the severity of the status.

P0278. Sisters with loss of methylation at both the TNDM and the BWS loci.

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Transient Neonatal Diabetes Mellitus (TNDM) is a type of diabetes presenting in the neonatal period. It is caused by aberrations within an imprinted region at 6q24. Three underlying genetic mechanisms are known so far: paternal uniparental disomy of chromosome 6, paternal duplication of 6q24, or loss of methylation (LOM) at the differentially methylated region (TNDM DMR) on 6q24. We present the first report of a family with recurrence of TNDM in two sisters with LOM at the TNDM DMR on 6q24. In addition to the LOM TNDM both sisters had partial LOM at the KCNQ1OT1 in the Beckwith-Wiedemann Syndrome (BWS) region on chromosome 11p15.5 (KvDMR, domain 2), as recently reported by us for the older sister (Mackay et al., 2006). The parents are first cousins. The clinical phenotypes of the girls show manifestations of both syndromes, albeit so far with macroglossia as the only BWS feature in the younger sister in whom the level of the KCNQ1OT1 LOM is smaller compared to her older sister. We suggest that these sisters represent a novel, most likely autosomally recessive inherited defect of the methylation machinery affecting at least two different loci. The present report has two important clinical implications: Firstly, the detection of concomitant KCNQ1OT1 LOM in these patients with LOM TNDM prompts for testing for KCNQ1OT1 LOM in future patients with LOM TNDM, as the molecular diagnosis of BWS may have implications for the clinical management including renal ultrasonography. Secondly, the recurrence reported in this family has implications for genetic counselling.

P0279. A triple X syndrome patient with anaemia and autoimmune disorder

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The 47,XXX is a sex chromosome anomaly that occurs in about 1 in 1,000 female babies.
Girls with triple X syndrome are sometimes taller than average and have an increased risk of learning disabilities and delayed speech; developmental delay and behavioural problems are not common. Most females with triple X syndrome have normal sexual development and are able to conceive children.
A case of a 20-year-old woman with a triple X syndrome is discussed.
The girl was admitted to the Haematologic Dept. because of asthenia and skin pallor. A severe not carential nor haemolitic macrocytic anaemia (Hb = 5.2g/dL; MCV = 112) was diagnosed. The bone marrow examination showed myelodysplasia. The negative DEB test allowed to exclude Fanconi Anaemia while a constitutional triple X karyotype was found.
Abdomen ultrasonography and oesophagogastroduodenoscopy were negative. The biochemical parameters resulted within the normal range, while anti DNA and anti nucleus antibodies were positive. An autoimmune connective tissue disorder was then hypothesized.
The anaemia origin is unclear. Moreover, chronic pure red cell aplasia, that in literature is known to occur on an autoimmune basis, was reported in a triple X Japanese woman, while thrombocytopenia was reported in few other XXX patients. Then a question about the correlation between chromosomal aberration, haematopoietic imbalance, and autoimmune disorder arises.
In addition, the karyotype analysis seems mandatory in women with inexplicable anaemia or thrombocytopenia and/or autoimmune abnormalities, even though phenotypically normal.

P0280. Three siblings of partial trisomy 19q13.3-qter together with monosomy 20q13.3-qter due to rare the maternal translocation t(19;20)(q13.3;q13.3)

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We present natural histories of three siblings at the age of 21, 19 and 13 years with the same unbalanced translocation in the form of the trisomy 19q13.3-qter with monosomy 20q13.3-qter unknown so far.Translocation was inherited from the mother, the carrier of t(19;20)(q13.3;q13.3) GTG, RBG. A study of morfological phenotype was performed according to Stengel-Rutkowski. A catalogue of well-defined traits was used for systematic collection of clinical symptoms and anthropological traits. Anamnestic data regarding preconceptional period, pregnancy, birth, neonatal period, developmental course and history of diseases were obtained from the parents and hospital records. A semi-standardised protocol was used for the assessment of rare anthropological traits in the skull, face, trunk and limbs. Facial measurements were performed from frontal and profile photographs quantifying seventeen traits by age related indices. On the basis of these data the child's trait list was set up by checking all informative catalogue features for presence or absence. Quantitative features analysis showed that a total 95 out of 807 (12%) of catalogued anamnestic and morphological traits were present in all children and only six additional features were distinct. Interestingly that some dysmorphic features (slightly asymetric, broad face, slopping forhead, upslanting palpebral fissures, long back of the nose, big nares, long philtrum, pointed chin, hipomimia) have been seen in the mother.

P0281. Genetic susceptibility to tuberculosis in Japanese: a gene-based analysis

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Tuberculosis (TB) is the second commonest cause of death from infectious disease after HIV/AIDS worldwide. Association studies have revealed that host genetic factors, such as HLA-DR2 and SLC11A1 (NRAMP1), play roles in susceptibility to TB. Recently, a linkage analysis on sib-pairs mapped susceptibility loci to chromosomes 15q11-13 and Xq26. To identify host genetic factors involved in the susceptibility to TB in Japanese, we performed a gene-based association analysis of 21 candidate genes [SLC11A1 (NRAMP1), vitamin D receptor (R), IL-1beta, IFN-gamma, IFN-gammaR1, IFN-gammaR2, IL-12 (p40), IL-12 (p35), IL-12Rbeta1, IL-12Rbeta2, STAT-1, IL-18, IL-18R, IL-23 (p19), IL-23R, IL-27 (p28), IL-27R (WSX-1), TNF-alpha, TNFRSF1A, TNFRSF1B and UBE3A genes] on 87 TB patients and 265 controls using marker single nucleotide polymorphisms (SNPs). For the genes with two or more marker SNPs exhibiting significant allele association, we subsequently analyzed the association between adjacent coding SNPs (cSNPs) and TB. Among a total of 118 marker SNPs, 3 of *IL1B* and 2 of *IL12RB1* showed association with TB. Non-synomymous cSNPs were not identified in *IL1B*. Association studies on 4 non-synomymous cSNPs of *IL12RB1* (641A/G, 1094T/C, 1132G/C, 1573G/A) in linkage disequilibrium showed that 3 of them were significantly associated with the development of TB. Haplotype analysis on the 4 cSNPs demonstrated that frequency of ATGG haplotype was significantly lower in TB patients than in controls. These data suggested that among the 21candidate genes analyzed, *IL12RB1* confers genetic susceptibility to TB in Japanese.

P0282. A severe virilized case with 45,X karyotype and cryptic Y sequence

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Approximately 50% of patients with TS have a 45, X karyotype, the rest have a structurally abnormal sex chromosome or are mosaic for a second sex chromosome. Molecular analysis has demostrated that some patient with TS have a cryptic Y chromosome mosaicism. The presence of Y sequences is correlated with risk of developing gonadoblastoma or dysgerminoma and require preventive removal of the gonads before hormone treatment.

We report on a 4-years old child with severe virilization 45,X karyotype and cryptic Y sequence. He was the first child of nonconsangineous parents and born at term. He had had right inguinal hernia repair before referral to our clinic. On physical examination weight was 13 500 g (3rd centile), height 94 cm (3rd centile). P

enoscrotal hypospadias, severe chordee, bifid scrotum, left nonpalpable testis were noticed. Ultrasonography showed vagina in the pelvis, and scrotal ultrasound demonstrated right testis with normal appearance, but left testis was not found. 45, X karyotype was found through cytogenetic study from peripheral blood lymphocytes and fibroblast of skin biopsy.

FISH analysis using SRY locus specific probe showed no SRY gene loci. Than, molecular genetic study was carried out using DYS14 "testis specific protein" localized to the Yp11.2 and SRY primers set by PCR. SRY was negative but DYS14 was detected positive in both DNA samples extracted from peripheral blood and testis tissue. Studies are underway to analyze other Y specific sequences

This is the most severe virilized patient who had 45,X karyotype and Y sequence reported so far.

P0283. The C1173T and G3730A polymorphisms in the vitamin K epoxide reductase gene and pharmacogenetics of warfarin in Russian population

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Warfarin is widely used for prevention of thromboembolic disease. There is evidence that the main cause of inter-individual sensitivity to warfarin determined by activity of the microsomal enzyme cytochrome P450 2C9 (CYP2C9) and vitamin K epoxide reductase (VKORC1). Recently, two polymorphisms C1173T in the intron 1 and G3730A in the 3'UTR of the VKORC1 gene were found. For revealing the allelic distribution of VKORC1 polymorphisms in Russian population 125 unrelated patients aged 15-75 years were investigated. In all patients the warfarin therapy was initiated with therapeutic INR target 2.0-3.5. For detection of the C1173T and G3730A variants of VKORC1 the polymerase chain reaction and original endonuclease digestion with HinfI and FauI were used. Our study showed that the frequencies of VKORC1 genotypes in Russian population were 46%, 38% and 16% for 1173CC, 1173CT and 1173TT, respectively and 37%, 50% and 13% for 3730GG, 3730GA and 3730AA, respectively. The 1173TT genotypes was associated with significant reductions in mean warfarin dose - 29.3±2.9 mg/week versus 42.3±2.3 mg/week for 1173CC and 40.1±2.6 mg/week for 1173CT ($p<0.05$). And this association was true when the contribution of the CYP2C9 gene polymorphisms in inter-individual sensitivity to warfarin was considered. No effect of G3730A on warfarin dose was detected in our population. In conclusion, the C1173T VKORC1 gene polymorphism may modulate the warfarin dose prescribed to acquire the target anticoagulation intensity and investigation of this genetic variant has important clinical significance in selection and individualization of warfarin dosage along with the CYP2C9 gene polymorphism determination.

P0284. Clinical variability in Weaver syndrome

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We investigated clinical variability of five patients with Weaver syndrome. All of the cases were referred to genetics department with unusual facies and developmental delay. Dysmorphic facies with hypertelorism, depressed nasal bridge, large ears, long philtrum and retromicrognathia were present in all patients. Macrosomia and accelerated growth of prenatal onset was significant in four patients. Two patients had congenital cardiac defects, developmental delay was present in all patients and mental retardation could be documented in three patients. Skeletal findings are also significant in this syndrome. All of our cases showed large bifrontal diameter and limited joint mobility. One patient also had other skeletal findings as camptodactyly, pectus carinatus, kyphoscoliosis, clubfoot and overriding of toes; another

patient had significantly limited elbow and knee extention. Loose skin, thin hair, thin-deep set nails, epicanthal folds and inverted nipples are occasional findings in Weaver syndrome and were present in one of our cases. Weaver syndrome is characterised by macrosomia, accelerated skeletal maturation, unusual craniofacial appearance, delayed motor and mental development, abnormal skeletal findings and looseness of skin. Sotos syndrome and other overgrowth syndromes should be included in the differential diagnosis. NSD1 gene mutations are described in both Weaver and Sotos syndromes; although, each syndrome has its own distinctive facial appearance.

P0285. Weill-Marchesani Syndrome (WMS) presenting as a rheumatologic condition

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Weill-Marchesani syndrome (WMS) is a rare condition characterized by short stature, brachydactyly, joint stiffness, and characteristic eye abnormalities including microspherophakia, ectopia of lens, severe myopia, and glaucoma. Both autosomal recessive (AR) and autosomal dominant (AD) modes of inheritance have been described for WMS. In our paper we report a case of WMS in a 12 year olds girls with history of myopia and operation on her eyes due to glaucoma in 8 years of age. She referred to us due to 2 years background of joint problems including stiffness of interphalangeal and intercarpal joints of her hands mimicking rheumatoid arthritis (RA). In physical examination she showed indurated skin of hands with remarkable limitation of motion in small joints of hands. Radiographic and laboratory studies failed to show any characteristic findings usually found in RA.

P0286. A nonsense mutation in the *Elastin* gene is responsible for Familial Supravalvular Aortic Stenosis

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Congenital Supra Valvular Aortic Stenosis (SVAS) is often part of a phenotypic spectrum encountered in the microdeletion disorder Williams syndrome (WS). Sporadic and/or familial isolated cardiac SVAS can also occur as an inherited autosomal dominant trait due to mutations (point, translocations, deletions) that disrupt the *Elastin* gene (*ELN*) on 7q11.23.

We describe a Caucasian family with isolated SVAS detected in a father and daughter. The daughter, a first child from unrelated parents, presented with cardiac arrest in the emergency room and eventually died. Investigations into the family history allowed us to identify an affected father who developed SVAS and was successfully operated on during childhood. There were no other phenotypes associated with WS, such as facial dysmorphism, stellae iridae, hypercalcemia or mental retardation, throughout this pedigree indicating that their condition was non-syndromic SVAS. Mutation screening (SSCP/heteroduplex analysis) of the *ELN* gene from both individuals identified a heterozygous nonsense mutation in exon 14 which leads to premature termination of the elastin transcript. The family can now be offered *ELN* mutation screening of future progeny.

This case underlines the pathological consequences of a point mutation in *ELN* gene and highlights the importance of family screening to improve the genetic counselling for future pregnancies.

P0287. Atypical 7q11.23 microdeletion sparing ELN and LIMK1 detected by aCGH in a patient with classical behavioral and dysmorphic features of Williams syndrome.

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Williams-Beuren syndrome (WBS, OMIM 194050) is a multisystem neurodevelopmental disorder associated in the majority of cases with a heterozygous deletion that involves ~25 contiguous genes within a ~1.5 MB region at 7q11.23. Molecular confirmation of WBS is routinely accomplished using a FISH probe for the elastin gene (ELN). The classic WBS phenotype consists of distinctive facial features, a cognitive profile including mental retardation with particular deficits in visuospatial abilities and relative sparing of verbal-linguistic function, a behavioral profile including both increased anxiety and highly sociable personality, elastin arteriopathy with supravalvular aortic stenosis, connective tissue abnormalities and mild growth retardation. Initially the search for the etiology of the WBS neurocognitive profile was centered on the LIMK1 gene, but more recently CYLN2, GTF2I, GTF2IRD1 and GTF2IRD2 have fallen under greater suspicion.

We describe a patient with the classical facial dysmorphic features and behavioral profile of WBS. Conspicuously absent was evidence of elastin arteriopathy, connective tissue abnormalities, growth or mental retardation. FISH testing with the common ELN probe revealed 2 normal signals. High-resolution aCGH using a tiling path BAC-array revealed a 2.7 MB deletion telomeric to but not including ELN and LIMK1. The boundaries of this deletion are presented along with further consideration of the genotype-phenotype implications raised by this novel atypical 7q11.23 microdeletion.

P0288. Familial overgrowth and Wilms tumor associated with paternally inherited duplication of IGF2 region on chromosome 11p15.5

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Overgrowth syndromes are a heterogeneous group of disorders. Associated features such as macrocephaly, hemihypertrophy, mental retardation, facial dysmorphism and congenital anomalies may help distinguish specific recognisable syndromes, such as Beckwith-Wiedemann Syndrome (BWS) However, the advent of molecular analysis has provided evidence for significant clinical and molecular overlap. Overgrowth syndromes are associated with increased risk of tumorigenesis.

We report a three-generation family with prenatal onset of a generalized overgrowth disorder. The proband (BW 5.8kg) presented with bilateral Wilms tumours at age 19 months. A younger sister (BW 5.08kg) was diagnosed with bilateral Wilms at age 5 months. The father (BW 5.5kg) had been treated for unilateral Wilms tumour at age 30 months. Intelligence is normal and there are no other characteristic features of BWS. High resolution karyotype and 11p subtelomere FISH were normal.

Three generations of the family were investigated. The results of clinical, cytogenetic (FISH using a cosmid IGF2 probe), and molecular analysis (genotyping of 11p with microsatellite markers; methylation studies at H19 locus; microarray analysis) are presented and are consistent with a submicroscopic duplication of the IGF2 gene region at 11p15.5 on the paternally inherited chromosome in affected individuals.

Loss of imprinting at the IGF2 locus (chromosome 11p15) has previously been implicated in a sub-group of patients with BWS suggesting that increased IGF2 gene dosage is important in the pathogenesis of the syndrome. This study identifies a novel mechanism and confirms others reports suggesting that increased IGF2 dosage is important in Wilms tumour predisposition.

P0289. Molecular genetics of the *ATPB7* gene in Russian Wilson disease patients

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Wilson disease (WD) is an autosomal recessive disorder characterized by dramatic build-up of intracellular hepatic copper with subsequent hepatic and neurologic abnormalities. WD is caused by mutation in the *ATP7B* gene (13q14.3-q21.1). We have investigated DNA samples from 144 unrelated WD patients for mutations in *ATP7B* gene 7 different exons by SSCP analysis. Altyered mobility fragments were followed by sequencing. We have revealed 6 different mutations in homo- and heterozygous state in 69 (48%) probands and of these,

28,4% were due to the H1069Q mutation. This mutation is the most frequent in Russian WD patients. Also other mutations such as c3400delC, c1744_1745delAT, c1770insT, delVal1217-Leu1218 and H1207R were detected and their frequencies amount 2.1%, 0.35%, 1.04%, 0.35% and 0.35% correspondently. The mutation c1770insT was unknown previously. The investigation of all other *ATP7B* gene exons is in progress now.

P0290. Neurological Sequels in Haemophilia Patients

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Introduction. Hemophilia is a X-linked inherited coagulopathy, due to mutations in either FVIII (haemophilia A) or FIX (haemophilia B) genes. Without adequate therapy, bleeding within the nervous system is the most frequent mortality and morbidity in haemophiliacs.

Objective. Starting from therapeutic deficiencies in Romania (prophylaxis absence, inadequate doses and duration of "on demand" therapy), we followed-up neurological manifestations and their sequels in haemophiliacs.

Material and method. Retrospective study based on 224 consecutive haemophilia A (84.38%) and B (15.62%) patients, registered and treated in Haemophilia Center Timisoara in a seven-year period. We followed-up: frequency of neurological manifestations according to haemophilia severity, pathogenic mechanism, age, inhibitor status, territorial distribution, evolution and type of sequels.

Results. An important proportion of patients (33.92%) presented bleedings with neurological symptoms, 38.15% of them having multiple symptomatic haemorrhagic manifestations. Both neurological complications (75%) and sequels (76.4%) were found especially in patients with severe haemophilia. Predominant pathogenic mechanism was nerve compression (62.71% of cases). 5.93% of neurological symptoms appeared in newborns. Patients with inhibitors presented a high risk of sequels. Most patients (52.94%) were from other counties, which leaded to a delay in therapy administration. From all patients with neurological manifestations, 44.73% presented sequels, the most frequent being central nervous system sequels, followed by peripheral neurological, sensorial and sensitive sequels

Conclusions. High frequency of the neurological manifestations and great rate of sequels advertise a sanitary enlightenment of the haemophiliac family for a rapid attendance of the patient, and optimizing of the diagnostic and the therapeutic attitude.

P0291. Clinical, biochemical, and DNA study of X-linked adrenoleukodystrophy in Russia

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X-linked adrenoleucodystrophy (X-ALD) is a relatively common and worldwide spread peroxisomal disorder with pronounced phenotypic variety. Clinical, genealogical, biochemical, and molecular genetic features of X-ALD in 35 families (54 patients) were studied. Childhood and juvenile cerebral forms predominate in the sample obviously due to incomplete diagnostics of adrenomyeloneuropathy and "pure" adrenal insufficiency, and in part, to a contingent referring for genetic counseling. In some families different phenotypes co-exist. Increase of very long chain fatty acids (VLCFA) is a reliable biochemical marker in all X-ALD phenotypes including asymptomatic stage. In 25 families molecular genetic study was performed, and 24 *ABCD1* mutations were found including 13 novel mutations. Only in two cases mutations occured de novo. Both VLCFA level and mutation type are not related to X-ALD phenotype and have no clinical prognostic value. Four asymptomatic cases in proband's junior brothers were revealed which is important in respect to early bone marrow transplantation. DNA diagnostics is more reliable for identification of female heterozygotes and for prenatal diagnostics, it is also less expensive and less laborious than biochemical investigation.

P0292. Identification of new genes and genetic mechanisms in patients with X-linked mental retardation

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In addition to traditional karyotyping, we have implemented array-CGH for the identification of disease-related genes and mechanisms in X-linked mental retardation (XLMR).

Allan-Herndon-Dudley syndrome (AHDS) is an X-linked MR syndrome that was only reported in males and is characterised by severe MR, neurological abnormalities and increased serum T3. Mutations in *MCT8* have been identified as the underlying genetic cause. Carrier females appear normal. Molecular characterisation of a balanced translocation t(X;9)(q13.2;p23) in a female patient with syndromic MR revealed the disruption of *MCT8.* Because of complete inactivation of the normal X chromosome, the expression of *MCT8* in the patient's fibroblasts is abolished. Therefore, this is the first female patient with AHDS.

Array-CGH allows genome-wide detection of submicroscopic copy number alterations in patients with idiopathic MR. We have developed and validated a full-coverage X-array for the detection of micro- and microduplications at high resolution in patients with suspected XLMR. Screening of 100 XLMR patients revealed genomic copy number alterations in 12% of the patients. These vary in size from a few 100 kb up to several Mb. In this way, we identified a small duplication at Xq28 in a large family with severe MR and progressive spasticity. Moreover, via genotype-phenotype correlation studies we could demonstrate that duplication of *MECP2* is the underlying cause of severe MR. This finding adds a new mechanism for MR-associated mutation of *MECP2* in particular and of XLMR genes in general.

P0293. XK aprosencephaly and 13q-deletion.

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XK aprosencephaly is a very rare malformation syndrome with atelencephalic microcephaly and limb anomalies. Autosomal recessive inheritance is suggested, however, an association with 13q-deletion is discussed in a few cases in the literature. Here we add a further case with the full blown malformation spectrum of XK aprosencephaly and 13q-deletion. The fetus (16+1 gestational weeks) had severe microcephaly with OFC of 8.5 cm and complete but hypoplastic neurocranium without bony closure defects as seen in anencephaly. The face was severely malformed with no nasal structure present. In addition, the fetus had gastroschisis with protrusion of the gut in the lower right quadrant, III-IV- syndactyly on the left hand, absent thumbs bilaterally and oligodactyly on the feet. The fetus had indifferent external genitalia, however, internal inspection showed uterus and adnexa. Except for hypoplastic pulmonal artery and hypoplastic ductus ateriosus, the internal organs appeared to be normal. Brain autopsy revealed agenesis of the cerebrum and cerebellum with well developed brain stem. Lymphocyte chromosomes in the mother showed an apparently balanced 4;13-translocation with breakpoints identified at 4q27 and 13q21. Micro-satellite analysis in the fetus using tissue block DNA to check for 13q-deletion revealed loss of the maternal allele at least at the locus D13S796 (13q33.3). Investigations at the loci D13S258, D13S631, and D13S258 were not completely informative, however, did not contradict loss of heterozygosity at 13q21.33-ter.

P0294. X-linked lissencephaly with agenesis of corpus callosum and ambiguous genitalia (XLAG), and intractable diarrhea- expanding of the clinical spectrum of XLAG

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Mutations in the aristaless-related homeobox gene (*ARX)* have been reported in non-syndromic X-linked mental retardation as well as in rare syndromes (X-linked lissencephaly with abnormal genitalia (XLAG), Partington syndrome (PRTS), and X-linked infantile spasm syndrome (ISSX)).

The patient is the second child of healthy nonconsanguineous parents and was born at term (W 2900g, L 49cm,OFC 33cm; karyotype

46,XY). The patient presented with intractable neonatal epilepsy. EEG showed burst suppression and multifocal hypersynchronous activity. MRI revealed lissencephaly/pachygyria and absent corpus callosum. Cerebellum and brainstem were normal. Recurrent episodes of hypothermia were interpreted as a sign of hypothalamic dysfunction.
The patient had ambiguous genitalia with a micropenis and an empty shallow scrotum, minor dysmorphisms, severe failure to thrive, feeding difficulties with poor sucking needing exclusively tube feeding. There was neither psychomotor development nor reaction to visual or acoustic stimuli. At the age of 3 weeks he developed intractable diarrhoea requiring parenteral nutrition. At 15 weeks of age weight was 4060g (P3), L 60cm (P25), OFC 34,3cm(< P 3).
Mutation analysis of exon 1-5 of the *ARX*-Gene revealed a deletion in exon 3 (c.1105delG) leading to a frameshift mutation with a consecutive premature stop of translation.
The severe phenotype is rare and mostly associated with frameshift/ premature truncation mutations. Intractable diarrhoea so far reported in only one case is of major clinical impact and might be explained by expression of *ARX* not only in the telencephalon, testes, and kidney, but also in the gut.

P0295. Identification and molecular modelling of a novel familial mutation in the *SRY* gene implicated in the pure gonadal dysgenesis

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SRY gene plays a key role in human sex determination and is responsible for initiating male sexual differentiation. The protein encoded by *SRY* contains a homeobox (HMG) domain, which is a DNA-binding domain present in some chromatin-associated proteins of the high mobility group family, and in some transcription factors. Mutations within the *SRY* open reading frame are reported to be associated with XY pure gonadal dysgenesis. The majority of these are *de novo* mutations affecting only one individual in a family. Only a small subset of mutations is shared between the father and one or more of his children. Most of these familial mutations are localized within the HMG box and only two are at the N-terminal domain of the *SRY* protein. Herein, we describe a young girl with pure gonadal dysgenesis and her phenotypically and fertile father carrying a novel familial mutation in the *SRY* gene at codon number 3. This mutation is resulting in a serine (S) to leucine (L) substitution in the protein. The secondary structure of the SRY protein was carried out by protein modelling studies. This analysis suggests, with high probability, that the N-terminal domain of the *SRY* protein, where we found the mutation, could form an α-helix from amino acid in position 2 to amino acid in position 13. The secondary structure prediction and the chemical properties of serine to leucine substitution stands for a potential disruption of this N-terminal α-helix in the *SRY* protein. This mutation could play a major role in destabilizing the interaction of the SRY protein with the target DNA or could favour a translocation of the *SRY* protein in a cellular compartment where could not play its DNA binding function.

P0296. Cryptic chromosome anomalies in 43 out of 220 subjects with MCA/MR: suggested diagnostic strategy.

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Mental retardation is a common disorder, affecting 2-3% of the general population and several studies point at chromosome imbalance as a major cause.
We analysed a total of 220 mentally retarded subjects, selected on the basis of a “chromosomal” phenotype. At the time of referral, all of them had already had a reportedly normal chromosome analysis.
Genetic analyses included 1) repeated conventional chromosome analysis, 2) telomere analysis by FISH, 3) locus-specific FISH, on clinical indication, 4) quantitative molecular cytogenetics in all positive cases, 5) microarray-CGH (preliminary analysis of 5 patients).
Chromosome anomalies were detected in 43 subjects (20%). The yield of the repeated conventional chromosome analysis alone was 7 % (16 anomalies). Interestingly, 4 of them were interstitial, and telomere analysis gave apparently normal results. The yield of telomere FISH analysis was 10 % (21 rearrangements). A total of 6 rearrangements were cryptic interstitial: 4 chromosome deletions were inferred by the presence of locus-specific tumors (retinoblastoma, FAP, non-polyposic colon cancer) and were confirmed by FISH; one case with 3q13 deletion and one case with 1p21.3 deletion were diagnosed by 1 Mb microarray-CGH.
Chromosome imbalances were distinctively associated with: mental retardation of any degree; growth anomalies, usually in form of disproportion between height, weight and head circumference (not true overgrowth or growth delay), minor physical anomalies (the strongest indicator), including hands and feet abnormalities, anomalous dermatoglyphics, in particular excess of arches, “gestalt” for chromosome anomaly.
New suggestions are provided in the diagnostic strategies of mental retardation. Genotype-phenotype correlations are discussed.

Po02. Cytogenetics

P0297. Chromosomal analysis in mental retarded children born with dismorphogenic features at Genetic Centre, GMCH, Chandigarh

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Here we report ten cases of Down’s syndrome with trisomy 21 chromosome, referred by the Pediatric department with mongoloid features muscular hypotonea, brachycephaly, protruding tongue, small low set ears upward sloping paplpebral fissures, single palmar crease, flat nasal bridge. It is noteworthy here that one case of Klinefelter’s syndrome with 47, XXY karyotype is also observed out of five children having dimorphic features at birth. The affected individual is 18 days old baby with the features of low set ears malformed right sided pinna, high arched palate, abnormal cry, wide spaced nipple, tuft of hair at sacral region, sandal gap in right foot, short neck and ambiguousgenitalia. In addition, we have also done karyotyping of 20 mentally retarded children, referred from mental retardation centre, GMRC, Chandigarh. Five cases were diagnosed with 21 trisomy, one child with mosaicism, one child with Edward’s syndrome. Rest of the children were found having normal karyotyping, indicating other biochemical metabolic defects.

P0298. High Incidence of robertsonian translocation in infertile males

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Robertsonian translocation are the most common structural rearragement of human chromosomes occurring at a rate of 1/1000 live births. 60% inherit the rearrangement from one of their parents and 40% occur denovo. This proportion rises to 1% in infertile men & has been associated with infertility or oligospermia. Among all possible types of Robertsonian translocation, the D/D class is the most frequent, & studies of both spontaneous abortions & live births, indicates a predominance of 13:14 translocations. Our finding of this chromosomal aberrations (3.7%) in the infertile males are in good agreement with literature of 3.3%. We found three men out of eighty infertile males with 13q14q fusion. These translocations can be transmitted via ICSI/ART. Although robertsonian translocation is likely to be found in chromosomes investigation of infertile men, their role in oligospermia is not clear. The testicular histology of the men carrying such a rearrangement shows a variable picture, ranging from severe impairment to near normality. Individuals carrying each of the ten possible nonhomologous robertsonian translocations of the five humans acrocentric chromosomes (13,14,15,21,& 22) have been reported, but two combinations, rob(13;14) & rob (14;21) are observed at a frequency than the rest (73% & 10)%,respectively, of all robertsonian chromosomes. Thus there is a need to screen infertile men prior to ART as it may result in birth of offspring with same anomaly and or may result in poor blasotocyst development and implantation failure.

P0299. A girl with partial trisomy 12p due to an unbalanced 5p:12p translocation

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We report a 7 months old girl with partial deletion of short arm of chromosome 5 and partial trisomy of short arm of chromosome 12. Her mother is a carrier of a balanced translocation between chromosomes 5 and 12 [46,XX,t(5;12)(p15.3;p12.2]. She had hypertelorism, flat face with midfacial hypoplasia, anteverted nostrils, thick alae nasi, low set ears with helical hypoplasia of right ear, carp-shaped mouth with everted lower lip, downwards slanting corners of mouth and short neck. She also had hypotonisity, developmental delay, hypothyroidism, mild mitral regurgitation, seizures, retinal abnormalities and atopic dermatitis. Clinical findings of 5 p deletion syndrome are not present, while her facial appearance and other findings are similar to trisomy 12p syndrome. Although; hypotonisity, seizure history and facial dysmorphic findings are previously described in trisomy 12p syndrome, hypothyroidism, retinal abnormalities and atopic dermatitis have not been described until today.

P0300. Molecular characterization of terminal 14q32 deletions in two children

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The occurrence of deletions on chromosome 14 is rare. Indeed twenty eight cases have been reported with distal 14q deletion diagnosed by conventional cytogenetics. Among them, five reports dealt with terminal 14q deletion (14q32 to 14qter). Four of them have been mapped by FISH or microsatellite polymorphism analysis. We report here the results of the physical FISH mapping in two patients with terminal 14q32-qter deletions. Both patients were mentally retarded and 14q deletion was diagnosed by systematic postnatal screening of subtelomeric regions. They presented with thick nuchal skinfold during pregnancy and had had a normal prenatal karyotype. They had also a short corpus callosum. The first patient was born at terme, BW: 3450 g, BL: 49.5 cm, OFC 35cm, he had feeding difficulties, gastro-esophageal reflux, with dysmorphic features: narrow face, bulging forehead, narrow, upslanted palpebral fissures, epicanthal folds, nose with upturned tip, long philtrum, narrow mouth, microretrognathia, and prominent earlobes. Inguinal herniae were surgically corrected. The second patient was delivered at terme. BW: 3.170kg, BL: 50cm and OFC: 33.5cm. He had an hypospadias, a bilateral blepharophimosis and ptosis, an ostium secundum interauricular communication and facial dysmorphic features: hypertelorism, long philtrum, tented upper lip, long palpebral fissures, and forehead hirsutism. At age 6 months, he showed infantile spasms. Cortical atrophy and hypoplasia of corpus callosum were then evidenced on MRI. The size of the deletions were established to be 5.5 Mbp long for the first case, and 4,6Mbp long for the second case.

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P0301. A new case of terminal 17q deletion

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We present the case of patient, SI, 2 years and 9 months old. She is the second child of unrelated, healthy and young parents. The pregnancy has a normal evolution.

She was born at term by normal delivery.

At the clinical examination we can observe a plurimalformative syndrome with dysmorphic face, microcephaly, abnormal dermatoglyphics, hypotony and severe mental retardation.

The complementary exam reveals supplementary spleen, spastic tetraparesis, squint and normal brain (on the CT). The complexity of the plurimalformative syndrome guide us to make the karyotype. The G-band karyotype of the patient was 46,XX,del(17q25-qter). The parents' G-band karyotypes were normal.

We wanted to mention that this deletion was not found on the search performed on Medline or Orphanet.

P0302. A detailed breakpoint analysis of 2 patients with rare 20q13.33 subtelomere deletion to define genotype/phenotype correlations

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Pure constitutional deletions restricted to the very distal band of the long arm of chromosome 20, i.e., 20q13.33 have never been reported so far. The 20q deletion syndrome is usually caused by a chromosomal deletion encompassing the region of 20q13.12 →20q13.32 and has features of growth retardation, severe malformations of the limbs, short neck, flat occiput, and mild facial dysmorphism All laboratories from the "Association des Cytogénéticiens de Langue Française" (http://www.eaclf.org) offering an appropriate cytogenetic service in France were surveyed over a 10-year period (1994-2004) for cases where a submicroscopic telomeric abnormality had been ascertained.

We report on the clinical phenotype of 2 patients with a de novo, isolated, subtelomeric 20q13.33 deletion. The deletion is associated in one child with global developmental delay, craniofacial dysmorphism, and in the other one with more severe mental retardation and autistic behaviour. Detailed breakpoint analysis in these 2 cases using FISH with bacterial artificial chromosome (BAC) probes, microsatellite and single nucleotide polymorphism (SNP) genotyping has identified a deleted region of approximately 1.33Mb, with a 324kb difference between the two deletions. At least 30 genes are deleted. The precise region of loss has been defined allowing us to identify genes that may contribute to the clinical phenotype through hemizygosity. Assignment of clinical features to specific breakpoints and refinement of predictive value may be useful in counselling.

P0303. A rare case of mosaic 22q11 microdeletion syndrome presenting with atypical features

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The acronym CATCH 22 (Cardiac Abnormality/abnormal facies, T cell deficit due to thymic hypoplasia, Cleft palate, Hypocalcaemia resulting from 22q11 deletions) has been used to describe the phenotypes of microdeletions within the band 22q11. The spectrum of clinical features varies from severe problems leading to early death in the neonatal period to subtle dysmorphic findings. Central nervous system malformations have been reported only in three percent of patients with this microdeletion.

We here report a mosaic case of 22q11.2 deletion who has atypical dysmorphic features and Dandy-Walker malformation which are all unusual for CATCH 22. Chromosome analysis at 550-600 band level revealed a normal karyogram. Due to congenital heart disease and frequent lower respiratory system infections, FISH analysis using D22S75 probe was performed and the deletion for this region was found in fifty percent of the peripheral blood cells.

The clinical, cytogenetical, and molecular cytogenetical findings of the index case and the parents will be presented.

P0304. Chromosomal anomalies found in patients referred with suspected 22q11.2 deletion: revisiting the clinical spectrum of DiGeorge syndrome

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Screening for 22q11.2 deletions is not an easy task because of the wide variability of the phenotype; many clinical features could overlap with those of other known syndromes and reported loci. Patients referred

to rule out a 22q11.2 deletion are usually tested with a locus-specific FISH probe, with a detection rate of around 10% depending on the selection criteria. Patients testing negative for FISH at 22q11.2 may have other chromosomal aberrations in routine cytogenetic analysis.
We have tested 533 patients suspected of having a 22q11.2 deletion. Fifty-seven of them (10.7%) were positive for 22q11.2 deletion, whereas 21 (3.9%) showed other chromosomal abnormalities involving deletions and duplications, marker chromosomes, apparently balanced and unbalanced translocations and sex chromosome aneuploidies.
This study, together with other previous reports, highlights the importance of initial conventional cytogenetic analysis in all referred patients, and provides useful data to optimize diagnosis and laboratory protocols according to the most frequent chromosomal findings.

P0305. Sex reversal and pseudo-hermaphroditism: The importance of gonadal mosaicism in females bearing a Y chromosome.

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Sex reversal and pseudo-hermaphroditism are rare phenomenons occurring in 1/20 000 individuals. Among the patients, many are phenotypic females with an intact or rearranged Y chromosome. Known genetic causes of these conditions include mutations of *SRY*, duplications of *DAX* and *WNT4*, deletions of *DMRT*, *WT1*, *SOX9*, or *SF-1*, or predominance of a 45,X cell line. We present here 10 cases of females with a Y chromosome : six isodicentric Y chromosomes, one ring Y chromosome, two XY females, and one XY individual with pseudo-hermaphroditism. Blood lymphocytes, skin fibroblasts and gonadal tissues were investigated by standard and/or molecular cytogenetics. The seven patients with a structurally abnormal Y chromosome were found to have a predominant 45,X cell line in blood and/or gonads, explaining their female phenotype. The patient with pseudo-hermaphroditism, who had a predominant 45,X cell line in her blood lymphocytes, had a predominant 45,X cell line in some structures of her gonads, but a predominant 46,XY cell line in the remaining gonadal structures, therefore explaining her ambiguous phenotype. The last two patients had an homogeneous 46,XY cell line in blood, and 46,XY/45,X mosaicism with a predominant 46,XY cell line in their gonads. Their phenotype is thus more probably explained by an infrastructural rearrangement of one of the genes implicated in sexual differentiation. These results therefore exemplify the necessity of cytogenetically investigating several tissues, especially the gonads, in order to explain the phenotype of these patients, and offer better cares and genetic counselling. *Support : RMGA-FRSQ and Fondation du CHU Sainte-Justine.*

P0306. An unusual case of simultaneous SLE and ALL in a nine year old girl.

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We present a case of a 9 year old girl, diagnosed with systemic lupus erythematosus (SLE). A bone marrow specimen was taken for further investigations which, on morphological examination, showed the presence of 10% lymphoblasts. Bone marrow immunophenotype analysis identified a population of cells consistent with a CD34+, CD79a, HLA-DR phenotype, accounting for 10% of the nucleated cells. Immunophenotype analysis also demonstrated a similar proportion of cells with a DNA index of 1.2. Cytogenetic investigations on bone marrow cultures revealed an abnormal high-hyperdiploid karyotype with gains of chromosomes X, 4, 6 and 10, two additional copies of chromosome 21 and an unbalanced der(16)t(1;16)(q21,q12) rearrangement in 4/50 (8%) of metaphase cells analysed. A locus specific probe for *AML*1 and enumeration probes for chromosomes X, 4, 6 and 10 confirmed the karyotype analysis. Loss of heterozygosity for *CBFB* was also detected in 18/200 (9%) of interphase cells. A diagnosis of acute lymphoblastic leukaemia (ALL) was made on the basis of the immunophenotype, cytogenetic and morphological findings. Concurrent ALL and SLE is a rare event, and this study addresses the possible significance of the der(16)t(1;16)(q21,q12) abnormality in this case.

P0307. Amniotic fluid alpha feto protein levels in Down syndrome pregnancies and other chromosomal abnormalities

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Amniotic fluid AFP levels were studied in a total in 1678 pregnancies. Eighty seven percent of the 23 Down syndrome pregnancies have amniotic fluid AFP levels at or below the median and 26% are below 0.5 MOM compared with 62% and 8.2% percent of controls. Only thirteen percent of the Down syndrome pregnancies have amniotic fluid AFP levels above the median.
The mean MOM for Down syndrome cases was 0.75. There was no significant difference of MOMs of Down syndrome cases at different gestational ages. Seventy five percent of the Down syndrome cases had amniotic fluid AFP levels below the median at gestational age of less than 16 weeks, while 86% had amniotic fluid AFP levels below the median at gestational age of 16 weeks and above.
No such association was seen for other chromosomal abnormality including trisomy 18, 45X, marker chromosomes, balanced autosomal translocations , and unbalanced chromosomal abnormality.

P0308. Mosaic 45,X/46,X, idic (Yq)/46,X,+mar(Y)/ 47,X,idic(Y),+mar(Y) in a newborn with ambiguous genitalia - A case report and review

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Ambiguous genitalia in a newborn is strongly suggestive of an intersex disorder, and proper investigations should be planned immediately after birth. In some cases the presence of a palpable gonad in the scrotum may induce to assign the male sex, whereas the anatomy of internal and external genitalia could be extremely complex. 45, X/46, X, idic(Yq) mosaicism is associated with a variety of phenotypic manifestations, including Ullrich-Turner syndrome (UTS), inter-sexuality, and complete male. A newborn with ambiguous external genitalia, (Clitoromegaly, Hypospadias, undescended testes) was investigated for assignment of gender. Chromosome analysis from peripheral blood culture showed, mos 45,X/46,X,isodic(Y) chromosome with predominantly 45,X cell line and instability of the Y chromosome. FISH analysis was carried out using CEP, X-Y and WCP-Y confirming standard cytogenetics studies. However, CEP XY showed in many interphase nuclei, a single X and Y centromere in majority of the cells. CEP Y signals varied from 0-3 confirming clonal mosaicism for Y chromosome and mono-centric and di-centric iso-Yq or iso-Yp. These findings suggested that the coexisting 45, X cell line is more influential on the determination of the sex phenotype in individuals with 45,X/ 46,X,idic(Yq) mosaicism. A careful evaluation of these patients is important at birth by a multispeciality team, for appropriate sex assignment and for the assessment of the risk of neoplastic degeneration should be considered.Possible mechanisms of formation of abnormal Y chromosomes and karyotype-phenotype correlations are discussed.

P0309. Pregnancy outcome of 513 cases of Amniotic fluid cultures

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Pregnancy outcome of 513 amniotic fluid cultures referred to this center because of high risk pregnancy (high maternal age, abnormality in sonography, fetal loss, history of offspring with psychomotor retardation, chromosomal abnormality in offspring…..) was studied. Chromosomal study of this group resulted in 16 abnormal karyotypes, 5 balanced translocations, and 492 normal karyotypes. All 16 cases with abnormal karyotypes, opted for therapeutic abortions. Two pregnancies with neural tube defects and one with thalassemia major were also therapeutically aborted. The remaining 494 cases with normal karyotypes and balanced translocation resulted in 442 term live-born healthy infants, 27 premature births, 14 IUFD and 11 abortions.

The karyotype results were compatible with the pregnancy outcome in all cases. For normal amniotic fluid chromosome results, absence of gross abnormality and concordance of the baby's gender with the sex chromosome reported from the amniotic fluid was considered as a correct result. From the 11 aborted cases 6 was preceded by leakage and/or hemorrhage. In our study the abortion rate (2.2 percent) is higher than the expected 1 percent. We believe it to be due to the high rate of genetic disease in our studied population. The high rate of consanguinity in couples with abortion and IUFD (63 percent and 35 percent respectively) and history of one of the following causes for referral: fetal loss, offspring with mental and/or psychomotor retardation, abnormality detected in sonography (45 percent and 43 percent) places this group in high risk category with causes for fetal loss other than chromosomal abnormality.

P0310. Comparative analysis of aneuploidy frequency in the resting and stimulated cells from workers upon exposure by harmful industrial factors

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Comparative analysis of aneuploidy frequency of the four autosomes (7, 11, 12, and 16) and sex chromosomes in the interphase nuclei of uncultured and cultured lymphocytes (PHA-stimulated) from workers of nuclear-chemical industry and clinically healthy men was performed by use of two-color fluorescent *in situ* hybridization. The total frequency of numerical aberrations of all the six chromosomes was similar in non-stimulated cells of both groups. At the same time the frequency of sex-chromosomes losses was higher in the test group. In cultured cells numerical chromosomal aberrations were scored after at least one cell division *in vitro*. Total frequency of aneuploidy in the cultured cells of individuals undergone to influence of the complex of harmful industrial factors has increased on average by 52 % in comparison with non-cultured ones (P=0.01). While in the control group a tendency of frequency increasing was observed only (23 %, P=0.25). Thus, influence of harmful industrial factors has caused more than double increase of a difference between frequency of aneuploid cells in cultured and non-cultured leukocytes in the test group in comparison with the control. We suggest that the expression of accumulated *in vivo* premutagenic damages of spindle apparatus takes place during cell division. These anomalies are manifested during cell division and result in numerical chromosomal aberrations. This circumstance must be taken into account as main principle for detection of the harmful influences causing numerical chromosomal aberrations.

P0311. Differentiation of Fanconi anemia from 'idiopathic' aplastic anemia by induced chromosomal breakage study using MMC and DEB

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Aplastic anemia (AA) is an inherited disorder in 20% of cases. Idiopathic AA, in whitch no cause is apperent, accounts for 65% of all cases of AA.

Fanconi anemia (FA) is a rare autosomal recessive disorder with an extensive genetic and clinical heterogeneity. The lymphocytes of FA patients show increased sensitivity to alkylating agents such as mitomycin C (MMC), generating increased chromosome breakage. Due to the variability of malformations in FA patients and the hematologic consequences of the disease, it turns out that fiablility of diagnosis is important and until now, it has been based upon cytogenetic studies of chromosomal instability.

This study was conducted to differentiate between FA and idiopathic AA. We have investigated cytogenetically 26 patients referred for AA. MMC with 2 different concentrations and DEB have been applied to the lymphocytes of the patients and normal controls. In our laboratory, cytogenetic diagnosis of FA is based on the combined analyses of several criteria. According to these criteria, only 12 cases were classified as FA patients. Cases with sinificantly more breaks than in normal controls, but less than FA range, are considered ambiguous. In these cases, treatment with DEB or MMC on fibroblasts can be induce elevation of chromosomal breakage and by this method it's possible to detect specifically the somatic mosaicism. MMC and DEB proved to be sensitive methods for diagnosis of FA. This diagnosis can be made unequivocally by combining both the clinical data and the cytogenetic evaluation of chromosomal breakage.

P0312. Inherited deletion of chromosome 21q might reveal a recessive trait for cheilognathopalatoschisis and nystagmus in a three-year-old boy.

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Developmental delay, congenital abnormalities and dysmorphic features are often related to genetic alterations. High-resolution techniques such as array comparative genomic hybridization (array-CGH) are increasing success rates in the search for genetic alterations in patients.

Here we present a three-year-old boy with developmental delay, a bilateral cheilognathopalatoschisis, nystagmus and an occipital black hair lock. Routine cytogenetic screening (GTG-banding) revealed no alterations. With array comparative genomic hybridization (array-CGH), using BAC/PAC arrays with a resolution of approximately 1 Mb, we detected a deletion on chromosome 21, ranging from q22.3 to qter. The estimated size was found to be between 0.5 to 2.1 Mb. Further analysis using a whole genome tiling path array revealed that the size of the deletion is at minimum 0.5 Mb and at maximum 0.75 Mb. The exact karyotype was defined as 46,XY,del(21)(q22.3). No similarities were seen between the phenotype of our patient and other described cases with 21q deletion. The deletion was found to be inherited from the phenotypically normal mother.

Because of the inherited nature of the deletion, we suggest that the phenotype of the patient is caused by recessive inheritance and that the father is an obligate carrier of a mutation in a gene in this region. Sequencing of three candidate genes is in progress.

P0313. Array-CGH in a series of 25 patients with mental retardation, dysmorphic features and congenital malformations revealed an interstitial 1p22.3-p31.1 deletion in a patient with Goldenhar-like syndrome

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Genosensor Array 300 (Abbott) is a multiplex platform for array-based comparative genomic hybridisation that detects unbalanced genomic aberrations including whole chromosome gains/losses, microdeletions, duplications and unbalanced sub-telomeric rearrangements. We analysed a series of 25 patients with unexplained mental retardation, dysmorphic features, congenital abnormalities and normal high resolution karyotype and FISH telomeric studies. Array-CGH identified one chromosomal aberration with an interstitial 1p31.1 deletion. CGH analysis confirmed the interstitial 1p22.3-p31.1 deletion. The patient was a 20-year-old boy with short stature (1m65), facial asymmetry, hypodontia, short neck, down slanting palpebral fissures, long nose, small mouth, severe scoliosis, moderate psychomotor delay and epibulbar dermoid. The phenotype was compatible with Goldenhar syndrome although the presence of mental retardation, absence of asymmetric ears and absence of deafness. Molecular bases of Goldenhar syndrome remains unknown. Most cases are sporadic but rare familial cases are reported following an autosomal dominant inheritance. Interstitial deletions involving the 1p22.3-p31.1 region are rare, only five cases have been reported with variable phenotype including hypertonia, micrognathia, short neck, proximal implantation of thumb, fifth finger clinodactyly, increased joint laxity and mental retardation. This observation is of interest since it could be a clue in the search of the genes responsible for Goldenhar syndrome. This study demonstrates the utility of the Array-CGH technology to detect interstitial deletions.

P0314. A familial duplication of Xp22.2 analysed with high resolution X chromosome specific array-MAPH (Multiplex Amplifiable Probe Hybridization) methodology.

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We report a family with syndromic X-linked mental retardation caused by a duplication of Xp22.2. This family has four mentally retarded sons (three are deceased) and one unaffected carrier daughter. Both parents are healthy and non-consanguineous. Clinical findings of the affected males include: hypotonia and developmental delay, scoliosis, cardiovascular problems and soft dysmorphic facial features. Speech problem, emotional disturbances and mental retardation were present in all but to varying degrees. Female carriers have normal phenotype and intelligence. Cytogenetic analysis of the mother showed an abnormal chromosome X with a possible duplication of Xp22.2 which was passed to all her offspring. FISH using whole chromosome paint confirmed the X origin of the duplication. Further characterization of the abnormal chromosome X was performed by FISH using Kallman (KAL) probe and several bacterial artificial chromosomes (BACs) covering the region Xp22.12-Xp22.3. Array-based multiplex amplifiable probe hybridization (array-MAPH, Patsalis et al. EJMG 2005; 48:241-9) using a full coverage (238Kb median spacing of probes) chromosome X specific array, confirmed both the presence and the location of the duplication (Xp22.2) and also revealed that the size of the duplication is approximately 9 Mb. Based on FISH and array-MAPH analyses the location of the duplication is between 9.78-18.83 Mb of chromosome X. Array-MAPH has been demonstrated to be a valuable tool to detect, characterize and confirm small chromosomal abnormalities. Furthermore, the clinical and molecular characterization of chromosome X small size deletions and duplications provides important information for accurate description of genetic syndromes, genotype-phenotype correlation and genetic counselling.

P0315. Investigation of sex chromosome abnormalities in patient‘s with azoospermia and oligospermia by standard Cytogenetics techniques and Interphase Fluorescence In Situ Hybridisation

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To investigate the prevalence of sex chromosome abnormalities among azoospermic and severe oligospermic men, a retrospective study was carried out in 515 infertile men .

All patients were subjected to lymphocyte culture and analysis of G-banded chromosomes. A minimum of 20 cells were analysed for each case. The samples suspicious of containing more than one cell line were subsequently studied by dual coloure interphase fluorescence in situ hybridization (FISH) using probes specific for centromeric region of chromosomes X and Y. The red (specific for chromosome X) and green (specific for chromosome Y) signals were enumerated on a minimum of 200 interphase cells .

68 patients showed an abnormal sex chromosome constitution using GTG-Banding. 54 pateints showed 47, XXY karyotype using both methods. Fourteen samples were mosaic for X chromosome aneuploiy by conventional cytogentic analysis. The subsequent interphase FISH study on these samples showed: Two red and one green signals in an average of 97% of hybridized cells in two samples, indicating for 47,XXY karyotype; one red and one green in 37% of cells with two red and one green signals in 63% of cells in 9 samples, revealing the mosaic karyotype of 46,XY/47,XXY. The remaining 3 samples showed three types of cell lines: 21% of the cells 46,XY, 53% of the cells 47,XXY and in 26% of the cells48,XXXY (46,XY/47,XXY/48,XXXY mosaic).

The results indicate that the intephase FISH has the advantage of detecting the mosaic form of aneuploidies.

P0316. Description of constitutional de novo balanced reciprocal translocation in patient with abnormal phenotype

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Balanced reciprocal translocations are one of the common structural chromosomal abnormalities and could be either without any phenotypic effects or with abnormal phenotype. In our registry of chromosomal rearrangements were ascertained 8 cases of balanced translocations. 5 of them were phenotypically normal individuals but had reproductive difficulties including recurrent miscarriages and/or offspring with congenital abnormalities due to segregation of an unbalanced form of the translocation. 3 carriers of reciprocal balanced translocations associated with multiple congenital anomalies including mental retardation and facial dysmorphology.

We examined an 18 years old boy with 46,XY,t(7;9)(q32;q22) karyotype which have numerous abnormalities. Nonconsanguineous parents had normal karyotypes so the translocation was *de novo* and look like as balanced. He was born at term after uneventful pregnancy and delivery. The patient had square cranium with prominent tubers. X-ray examination shows nonclosure median suture. He is noted to have thick eyebrows, wide forehead, cerebral region exceed the face, flattened downward nose, asymmetric by size and shape eyes, myopia 1.0, chronic conjunctivitis due to the barrier for tears outflow. He had calf teeth, some of them absent, diastema, hypertrophied and deformed gingiva. X-ray examination shows the absence of teeth’s rudiments. His ears are massive deformed and lowest. He had short neck, genu valgum limbs, bigtoe valgus deviation, II-IV drumstick toes, brachydactyly. The patient had very low level of parathormone: 2.0 (against 8,3 - 68). He had not mental retardation. This case requires further investigation of breakpoints mapping for searching cryptic imbalances or disrupted genes.

P0317. Co-localization of the region of genomic instability at distal 9p in *BRCA2* mutation carriers with the new fragile site *FRA(9)(p22.2)*

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Germ-line mutations in *BRCA2* gene (13q12-13) lead to a truncated protein, confer a high risk of breast cancer. *BRCA2* acts as a tumor suppressor gene and consistent with it, BRCA2 protein plays a significant role in maintaining genomic stability by involvement in DNA repair and recombination. In pursuit of finding out whether mutation in the *BRCA2* gene influences the genomic instability, the constitutional karyotypes of *BRCA2* heterozygous mutation carriers were analyzed. Previously, with the application of two-color FISH with YAC probes, we identified chromosomal rearrangements at 9p23-24 in three families with hereditary breast cancer. A common region of duplications, inversions and amplifications was established for them (Savelyeva et al., 2001).

Our further analysis of distal 9p region by multicolor FISH with different BAC clones allowed us to determine more precisely the map of rearrangements at the 9p region.

In search of a cause of this instability, we propose that unknown fragile site might be responsible for observed chromosomal aberrations. We induced fragile sites in lymphoblastoid cell lines of *BRCA2* mutation carriers by aphidicolin, which partially inhibits the DNA synthesis. This led to the identification and further detailed genomic localization of the new aphidicolin-inducible fragile site, *FRA(9)(p22.2)*. We show that *FRA(9)(p22.2)* breaks are limited to a 500 kb region.

The identification of *FRA(9)(p22.2)* rises the question whether this fragile site is commonly expressed in the human population or it is specific for the *BRCA2* mutation carriers.

P0318. Supernumerary marker chromosome detected in a fetus with a related pseudodicentric chromosome

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Dicentric autosomes are considered as unstable constitutional chromosomes in humans. The presence of centromeres on the same chromosome leads to a high risk of attachment of the same chromatid to the mitotic spindle from opposite poles and to the formation of anaphase bridge during cell division. Therefore, breakage of the dicentric can occur. We describe a fetus in which a minute supernumerary marker chromosome (SMC) was detected in addition to a larger pseudodicentri**c** chromosome. The case was a 12-week fetus with mosaicism for a normal and two abnormal cell lines: one had a dic (12;15)(q11.2;q11.2) chromosome the other a minute SMC. Deletion of centromeric material was proposed as one mechanism of centromere inactivation in dicentric chromosomes. This SMC may be the result of a deletion event leading to inactivation one centromere of a dicentric chromosome to generate a pseudodicentric chromosome. Therefore, these case suggest possible mechanisms for the origin of minute SMC.

P0319. Cytogenetic effects in the groups of high priority in different terms following the Chernobyl accident

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Selective cytogenetic monitoring among selected groups of high exposure (about 2000 persons) from the Chernobyl nuclear plant accident in Ukraine. Studies have been conducte since January 1987 till on patients recovered from acute radiation sickness, clean-up workers mainly from the 1986-1987 years, Chernobyl power plant personnel (including Sarcophagus workers), self-settlers from 30-km distance zone, children and adults from regions of obligatory and voluntary evacuation. The frequency of chromosome aberrations in human peripheral blood lymphocytes were determined with the help of conventional cytogenetics, G-banding analysis and FISH-WCP method. The data received during 10-15 years following the accident demonstrated a dose-depended increase of chromosome aberrations level in exposed groups and confirmed the growth of intensity of somatic chromosome mutagenesis induced by Chernobyl accident factors. It have been shown that even so called “low” radiation doses under prolonged exposure induce specific chromosome damages, which can serve not only as biomarkers of radiation exposure, but as indicators for selection of risk groups as regards to realize of harmful health effects with genetic component.

In order to study late effects (in 15-20 years) following the accident, new approaches for the evaluation of genetic consequences of human radiation exposure have been introduced. These include the study of so called “disgenomic effects” (especially delayed, hidden, transmissible chromosome instability and “bystander effect” both in exposed persons and in their progeny.

The results of selective cytogenetic monitoring suggest that the Chernobyl accident is an important ecogenetic factor for the population of Ukraine.

P0320. Results of FISH-WCP analysis in liquidators of Chernobyl accident including some oncohematology patients

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The important part of the Ukrainian-American Scientific Project “Study of leukemia and other hematological diseases among clean-up workers in Ukraine following the Chernobyl accident” is a retrospective individual dosimetry in Chernobyl liquidators with the help of several methods including FISH-WCP technique. This method had been used successfully for the reconstruction and verification of individual radiation doses in 231 clean-up workers including 5 liquidators with leukemia. The matched control group consisted of unexposed persons - 20 healthy males and 11 patients with oncohematological disorders. In civil liquidators cases with overestimated official doses predominated (mean group doses - 600 and 450 mGy, accordingly). In military liquidators cases with underestimation of official doses in comparison with FISH doses prevailed (mean group doses - 340 and 430 mGy, accordingly). According to results of FISH dosimetry both groups had been considered as “high-dose” with radiation exposure that exceeded permissible for radiation accident (250 mGy). In unexposed patients with leukemia the frequency of stable chromosome aberrations (excluding specific clones) did not depend on diagnosis and did not exceed control level. In all liquidators with leukemia the frequency of nonclonal reciprocal translocations were increased significantly in comparison with unexposed patients that in 4 cases corresponded to doses 310 - 400 mGy and in one case - 2240 mGy. It had been established that highest cytogenetic effect had been caused by previous radiation therapy that had been revealed by FISH analysis.

P0321. Clarifying the role of cell cycle control proteins Chk1 and ATR using cytogenetics and molecualr cytogenetics.

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ATR and CHK1 are proteins involved in cell cycle control. Their purported action, when there is slow-down or complete discontinuation of DNA synthesis at replicatively complicated sites in the genome, is thought to halt synthesis elsewhere in the genome. Examples of such sites are repeat sequences that allow DNA to twist into unconventional non-B DNA (non-helical) structures making synthesis difficult. These repeats are particularly prevalent at both common and rare fragile sites (FS); and are thought to be the antecedents of FS expression seen in classical Cytogenetics. ATR/CHK1 induced cessation of DNA synthesis allows replication machinery to reattach at these loci and proceed beyond them.

A mechanism to investigate the role of CHK1 and ATR could therefore be the potential increase in breakage at fragile site areas after knocking down the function of these proteins. We abrogated the normal in vivo function of ATR and CHK1 in HELA cells using siRNA, and investigated the effect on metaphase phenotype and fragile site stability. We have designed a dual-colour fusion probe spanning FRA16D and have found by cytogenetics and FISH that the increase in FS expression upon knock-down of the ATR/CHK1 pathway was marked. It corroborated evidence for both non-B DNA formation leading to replicative difficulty and for the putative functions of ATR and CHK1.

P0322. Frequency and spectrum chromosomal aberrations in somatic cells at the population living in a zone of influence of Semipalatinsk test site

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With the purpose of study cytogenetical effects in somatic cells at the population irradiated as a result of ground and atmospheric nuclear tests in 1949-1962 years. The research consist from complex cytogenetical analysis (G - method) 174 cultures lymphocytes in peripheral blood (66068 metaphases), including the analysis of frequency and various types chromosomal aberrations.

The greatest frequency of cells with aberrations is found out in a zone extreme radiation risk (ERR) -3,2±0,4 %, that in 2 times is higher, than in zone minimal radiation risk (MRR) - 1,8±0,2 % and in 3 times - in control area - 1,04±0,16 %.

In spectrum aberrations prevail acentric fragments, the frequency has made them in zone ERR 1,3±0,2, HRR - 0,94±0,13, that authentically differs from this parameter at the persons of zone MRR- (0,43±0,06) and control groups (0,2±0,06) on 100 cells accordingly.

The frequency dicentrics at the persons from ERR and HRR has made 0,18±0,03, rings - 0,27±0,06, that is higher, than at the persons from MRR and control group - 0,02±0,01 on 100 cells, accordingly.

The stable markers (deletions, translocations) radiating influence have made among surveyed from zone: ERR - 0,74±0,16, HRR -0,84±0,12, MRR- 0,63±0,13, in the control - 0,37±0,08 on 100 metaphases, accordingly.

Thus, the complex analysis of frequency and types chromosomal aberrations has shown, that in somatic cells of the inhabitants of the surveyed areas the tendency to accumulation of unstable and stable radiating markers is observed, that testifies to possible continuation of radiating influence on chromosomal the device.

P0323. Cytogenetic studies on the workers occupationally exposed to Cement dust

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Cement factories emit plenty of dust which is potential health hazard. It is composed of CaO, SiO_2, K_2O, SO_3, Al_2O_3, MgO and Fe_2O_3 in varying proportions. In addition, various brands of cement contain metals such as Mn, Cr, Cd, Ar, Pb, Zn, Cu and Ba. Prolonged exposure to cement dust causes specific mortality and tumor morbidity with the risk factor for right sided colon cancer and laryngeal cancer. In the human health arena, the consequences of exposure to genotoxic substances have been researched intensively for decades. Structural changes to the integrity of DNA caused by DNA-damaging agents are useful end point for assessing exposure to hazardous environmental pollutants on human health. Keeping this in mind, the present study is designed to analyze the effect of cement dust on the genetic materials of human, who are occupationally exposed to cement dust in the cement industries. By using peripheral blood leucocyte culture, of experimentals and controls, it is observed minor chromosomal aberrations such as chromatid gaps, breaks and fragments. From the data obtained, it is inferred that the age of the persons and the period of exposure of stone dust are directly proportional to the number of chromosomal aberrations. The data are discussed pertaining to the recent literature available.

P0324. Comparison of early and mid-trimester Amniocentesis in 1459 Amniotic fluid cultures

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Cytogenetic results of 655 amniotic fluid obtained at 12-14 gestational week (early amniocentesis) and 804 fluids at 15-18 gestational week (mid-trimester amniocentesis) were compared. The rate of chromosomal abnormalities for early amniocentesis was 3.2 percent and for mid-trimester amniocentesis was 5.3 percent and for level III mosaicism (EA = 0 percent, MA=0.24 percent) . Level I and II mosaicism occurred more frequently in mid-trimester amniocentesis. We did not have maternal cell contamination in either group. The number of repeat amniocentesis was 0.6 percent in the EA group compared with 0.1 percent in the MA group. Procedure-related abortion, leakage and hemorrhage after amniocentesis was not significantly different between these two groups 1.7 percent, 1.2 percent for EA and 0.9 percent 1 percent respectively. Follow-up of 508 of the above cases revealed sex compatibility with karyotype results in 100% of both groups. No gross abnormality such as equino-varus was detected in the live-born infants reported with normal karyotype in both groups.

P0325. Correlation between chromosomal abnormalities and Fertilization rate in infertile couples

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Introduction: The aim of this study was to determine the relationship between rate of chromosomal abnormalities in infertile men abnormal and out come of pregnancy rate (OPR).

Materials & Methods: It was retrospectively reviewed the genetic abnormalities detected clinically in 150 infertile men who were referred for their infertility problem. Semen analysis (SA) was performed on semen samples. Metaphase spreads were made using standard cytogenetic techniques. Cultures were harvested and Karyotyping was performed on G-banded chromosome preparations. The chromosomal status was analyzed using CytoVision Ultra ver.4.0 from Applied Imaging. The results of OPR were blindly reviewed. According to the normal and abnormal chromosomal analysis, they divided into two groups as 1 and 2. The OPR was compared between group using X^2 and the level of <0.05 was considered significantly different.

Results: SA was abnormal in all infertile men, mean $\pm$SD of sperm count was $14x10^6$/ml$\pm$2.1 and all were recommended to have ICSI treatment.

Of those patients 16 men had abnormal chromosome analysis. The Chromosomal analysis revealed that 10 samples analyzed as Klienfelter syndrome (47,XXY, n=5, and mosaic 47,XXY/46,XY, n=4, 47,XXYY n=1), the other were 46,XY, +mar (n=1), 46XY, dup; (4)(q31.1;q32)(n=2) , 46XY,Yqh+(n=2), 46,XY,del(13)(p)(n=1).

The biochemical PR test by β hCG revealed one pregnancy in group 1 (1/16, 6.25%), and 17 pregnancy in group 2 (17/105, 16.2%), p<0.01.

Conclusion: It could conclude that the chromosomal abnormalities in men can decrease the rate of pregnancy or later cause spontaneous abortion.

P0326. Premature ovarian failure resulting from a chromosome Xq28 deletion

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Premature ovarian failure (POF) is defined as a secondary hypergonadotropic amenorrhea occurring before 40 years old. It has been associated with environmental, autoimmune or genetic causes such as chromosome X deletions and translocations. Cytogenetic and molecular characterization of these abnormalities have led to the delineation of two Xq POF critical regions spanning respectively the Xq26-q28 (POF1) and the Xq13.3-q22 (POF2) chromosomal regions. In particular a recent study comparing chromosome X deletions observed in four women with POF allowed to define a 4Mb Xq28 POF1 critical region. Here we described the smallest chromosomal Xq28 deletion published to date. It confirms that POF1 critical region is located within the Xq28 band.

The patient is a 35 years old woman. She presents with POF and Hashimoto thyroiditis. No ovarian antibodies were detected.

A chromosome Xq28 deletion resulting from an unbalanced (X;Y)(q28;q11.2) translocation was detected on her karyotype. Molecular detected a 7,5Mb chromosome Xq28 band deletion. The mechanism involved in the aetiology of POF in our patient will be discussed:

P0327. Various outcomes of cytogenetic evaluation in patients with hypogonadism

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We report on diverse chromosomal findings in a series of patients referred to cytogenetic evaluation for hypogonadism or suspicion of sex chromosome abnormality during a period of 2 years.

Patient 1 is a 7-year-old boy with abnormal external genitalia: small penis, bilateral cryptorchidism and absent scrotums; testes were not palpable in the inguinal channels, nor found on abdominal NMR. He was moderately mentally retarded and had bilateral absence of patella. Chromosome analysis detected dup(1)(p22.1p31.1). Patient 2 is a 5-year-old boy with macrocephaly, mild facial dysmorphism, mild psychomotor delay, obesity, small penis, undescended testes and hypoplastis scrotums. Conventional cytogenetic analysis evidenced additional material on 12p. Patient 3, aged 25 was referred with suspicion of Klinefelter syndrome with tall, asthenic body habitus, varicosity of legs, mild learning disorder and recurrent depressive episodes. A mosaic karyotype: 46,XY/47,XXY/48,XXYY was found. Patient 4, aged 48 had gynecomastia, history of cryptorchidism, low testosterone, elevated FSH levels and a mosaic karyotype: 46,XY/46,XX/47,XXY. Patient 5 was also suspected of Klinefelter syndrome, with eunuchoid body habitus, gynecomastia, sparse body hair and high-pitched voice. Chromosome analysis revealed normal male karyotype; hormonal investigations showed low testosterone, FSH and LH levels; tentative diagnosis of Kallmann syndrome was made. Patient 6 aged 22, diagnosed with β-thalassemia minor in childhood, symptomatic, having required blood transfusions, presented with short stature, absent secondary sexual characteristics and primary amenorrhea. Hormonal evaluation revealead hypogonadotropic hypogonadism. Surprisingly, 45,X karyotype was found.

The cases presented above highlight the importance of cytogenetic examination in the evaluation of all patients with hypogonadism.

P0328. Individual sensitivity of chromosomes of human lymphocytes to radiation: stage-effect dependence.

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Prediction of human individual radiosensitivity on chromosomal level is relevant for revealing individuals with increased radiation and cancer risks as a correlation exists between genome instability and the probability of cancer development. This study presents the investigation of in vitro irradiation and individual cytogenetic effects in peripheral blood lymphocytes of 20 healthy donors on different stages of the cell cycle. With this purpose analysis of chromosomal aberrations in cultured lymphocytes after 1,5 Gy γ-irradiation in G_1, S and G_2 stages of cell cycle was carried out.

It was shown that chromosomal radiosensitivity varies qualitatively and quantitatively during the cell cycle. Transition of chromosome type aberrations to chromatid was marked during the progress of cells to mitosis. Exchange aberrations prevailed in the first half of the cycle whereas a change in damage spectrum to deletions in the second half of cycle was observed. The lowest chromosomal sensitivity to γ-radiation was observed in the S- stage and the highest in late G_1-stage and G_2-stage. Thus the highest variations in individual chromosomal sensitivity to radiation were detected in G_2-stage (from 14 to 124 aberrations/100 lymphocytes) in comparison with G_1-stage (from 56 to 83). High CV value 24% was observed for G_2 - radiosensitivity parameters. Analysis of the character of their distribution made it possible to reveal 12% individuals from investigated group with increased chromosomal radiosensitivity. The data obtained allow improving and recommending G_2-assay for cytogenetic monitoring of critical groups of population taking into account development of stochastic effects of Chernobyl accident in Ukraine.

P0329. Prenatal and Rapid Prenatal Diagnosis of trisomy 21 in Cultured and Uncultured Amniotic Fluid

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The aim of the present work was to examine the efficacy of using both conventional cultures from amniotic fluid and chorionic villi and FISH tests for rapid prenatal diagnosis of common chromosome aneuploidies. During one year were investigated 103 pregnant women by amniocentesis and 27 pregnant women by chorionic villi samples. A total of 10 pregnant women with Down syndrome fetuses over of ten months period were included in this study. Karyotypes obtained from amniotic fluid or chorionic villi, and FISH diagnosis was possible in all cases. A mosaic for trisomy 21 proved, by comparison with the extensive analysis of long term cultures to be positive. Otherwise the techniques were reliable, accurate and relatively straightforward to perform. Results of long term cultures were obtained in 12 - 14 days, while results by FISH could be available within 48 hours. In most cases an additional long term full analysis was also done, so to exclude rare aneuploidies and structural rearrangements. This methodology is seen as a useful addition to the prenatal diagnosis repertoire.

P0330. A supernumerary marker chromosome detected in a child with dysmorphic face and developmental delay

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A small C-band marker chromosome was detected on culture of blood lymphocytes of a child with flat nasal bridge, short palpebral fissures, micrognathia, high forehead, low-set ears. The fingers toes were abnormal. The child has developmental delay but with no sign of mental retardation. She suffers from sever anemia. The karyotype was done on peripheral white blood cells. Parental chromosomal analysis was done. Both parents had normal karyotype. Since the child was suffered form sever anemia, Fanconi test was done using standard protocol. However, the Fanconi test was negative. The origin of the marker chromosome is to be subsequently identified via chromosome microdissection and reverse-FISH.

P0331. Cytogenetic analyses of families with fertility problems

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Infertility affects approximately 15% of couples. It can be the result of chromosomal abnormalities in 2.8-13.1% of infertile couples: in females 1.1-9.8% and in males 2.1-14.3%. To assess the significance of chromosome anomalies in infertility, we have studied cytogenetically 90 infertile males (32 azoospermic and 58 oligozoospermic men), 25 infertile couples and 30 control fertile men. Chromosome analyses from peripheral blood lymphocytes cultures using GTG, C-banding and FISH methods were performed. Major chromosomal abnormalities showed 10-fold increase (13.4%) in 90 infertile males (15.6% in azoospermics and 12.1% in oligozoospermics), compared to the control group. There were sex chromosome abnormalities (47,XXY) in 5(5.6%) patients: in 4(12.5%) men with azoospermia and in one man with oligozoospermia. Autosomal structural rearrangements were found in 7(7.8%) patients, from which 6(10.3%) men had oligozoospermia. These results confirmed the high incidence of chromosome abnormalities in the infertile population. Both the numerical sex chromosome abnormalities and structural autosomal anomalies may cause the variable degree of spermatogenic disturbances.

We have studied 25 infertile couples, in which men had azoospermia or severe oligozoospermia (sperm count <5M/ml). Of these couples, chromosome anomalies were found in 6(24%): in 4 males (47,XXY) and in 3 females. In one couple, both male and female had chromosomal abnormality: 47,XXY/46,XY and 46,Xfra(X)(27.3), respectively. Therefore, we agree with authors, who suggest that chromosome analyses should be performed routinely in both male and female of every infertile couple. Supported by a grant from ESF number 5486 and target grant TARMP0421.

P0332. Chromosome 22q13.3 deletion syndrome with a de novo apparently balanced t(17;22)(q11;q13.3)

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The terminal 22q13.3 deletion syndrome is characterized by severe expressive language delay, mild mental retardation, and minor dysmorphisms. A number of different chromosomal aberrations have been found to be associated with this phenotype. Here we report a 3-years-old girl with an apparently balanced de novo translocation, t(17;22)(q11;q13.3). Clinically she demonstrates severe speech delay, mild mental retardation, and various dysmorphic features, such as high prominent forehead, epicanthic folds, strabismus, blue sclera, depressed nasal bridge, thin upper lip, prominent philtrum, malocclusion of teeth, large ears, narrow upper chest, narrow and sloping shoulders, skin dimples, clinodactyly of toes, and hyperflexibility of joints. She is considerably hypotonic since birth. We performed FISH analysis using numerous BAC clones to delineate both breakpoint regions of the 17;22 translocation and identified a 5-Mb terminal deletion comprising the chromosomal region 22q13.31-qter. These data suggest that this aberration is most likely associated with the majority of the phenotypic abnormalities seen in the patient, thereby further contributing to the clinical and genetic delineation of the terminal 22q13.3 deletion syndrome.

P0333. The analysis of 4p chromosomal abnormalities using micro-array CGH; genotype-phenotype correlation.

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The Wolf-Hirschhorn syndrome is usually caused by terminal deletions of the short arm of chromosome 4 and is phenotypically defined by growth and mental retardation, seizures, and craniofacial manifestations like the typical Greek warrior helmet. Large variation is observed in phenotypic expression of these features with mental retardation ranging from severe to mild.

To generate a phenotypic map of the region, we localized the breakpoints of the 4pter aberrations using a chromosome 4 specific micro-array, containing 1903 BAC/PAC clones from CHORI.

In total, DNA from a group of 25 WHS patients was analysed, 13 with a cytogenetic visible and 12 with a submicroscopic deletion. Six patients

carried an interstitial deletion, and three patients carried a deletion that did not include the Wolf-Hirschhorn critical region.
The correlation of the phenotypes of these patients with their genotype enabled us to refine the phenotypic map of the region. The deletion of the WHS candidate gene1 (WHSC1) is hypothesized to be the cause of the WHS facial characteristics. In contrast to this hypothesis, we identified one patient with the WHSC1 deletion, but without the facial characteristics, and one patient with the typical facies without the WHSC1 deletion. In the latter, a 500 kb upstream deletion was detected. Possibly, a positional effect influences the WHSC1 expression.
In conclusion, we show that small terminal 4p deletions encompass a spectrum of phenotypes, of which the WHS is the most common.

P0334. Phenotypic abnormalities found a case with chromosome deletion at 11q23.2: a case report

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An Iranian pediatric patient was referred to our clinic with some of the main phenotypic characteristics features of Turner syndrome. In addition, she had other morphological abnormalities such as prominent epicanthal folds, broad flat nasal bridge with short, upturned nose, short philtrum with carp-shaped mouth, and nonprogressive moderate psychomotor developmental delay. She has marked hypotonia. She had poor vision, hearing and speech problems. In addition, she was mentally retarded. Chromosome analysis was performed on her metaphases. All cells examined showed deletion in the long arm of chromosome 11. The karyotype of the child was reported as: 46,XX,del(11)(q23.2). The both parents had normal chromosome compositions. The phenotype of this case and other reported cases may help to define a syndrome caused by the deletion of this region.

P0335. High resolution cytogenetic assessment in 30 mestizos with Systemic Lupus Erythematosus

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SLE have been reported in association with Klinefelter´s syndrome and karyotypes 47 XXX. We reported chromosome changes in SLE patients in an early publication in 1982. We decided to performed Cytogenetic (CG) studies in 30 untreated venezuelan mestizos SLE-ACR criteria patients (26 females and 4 males, aged 16 to 60 years), to establish the prevalence of chromosome changes. In conventional and high resolution chromosomes, 1,500 metaphases and early prometaphases were studied in these patients and in 30 healthy controls matched by age and sex. Numerical and structural changes were seen in SLE group only (p: <0.001). Chromosome instability in the form of breakages in all the patients in more than 60% of the metaphases, being more affected Cr 1p, 6p, 12p and 14q; clonal changes in 104 (7 %) of the metaphases (-2, -12, + X (XXX, XXY) and -9,-10,-11,-13,-19,-20 clonal changes. Polyploidies and endorreduplications in 60% and 46.6% respectively; acrocentric segregation type GG and DG in 50% of the patients and genetic polymorphism in centromeric region type 1qh+, 9qh+ and 16qh+ in 20% of the patients.
We conclude that chromosome changes could be frequently seen in patients with SLE similar to those described in hereditary photosensitivity disorders and other conditions characterized by DNA damage repair defects and genetic instability such as in cancer and secondary to viruses and mutagenic agents. Prospective studies are required to define genetic polimorphism and also the genetic or mutagenic basis of these changes in SLE patients.

P0336. Application of chromosome microsurgery in producing FISH probes

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One of the advanced technologies in molecular cytogenetics is chromosome microsurgery. With the help of this technology, molecular genetics and cytogenetics come together in order to get more information about chromosome or part of a chromosome. In chromosome microsurgery the chromosome of interest or chromosomal region of interest is dissected using a fine glass microneedle under an inverted microscope. The chromosomal DNA isolated by chromosome microsurgery is then amplified employing one of the molecular methods such as PCR. The PCR amplified chromosomal DNA can be used in different applications for example fluorescent in situ hybridization (FISH).
In FISH, fluorescently labelled nucleic acid molecules are deposited in chromatin at the site of specific DNA sequences. By this method unique sequence, chromosomal subregions, or entire genomes can be specifically highlighted in metaphase or interphase cells. The technique is simple in principle. Specific DNA or RNA sequences are first labelled with nonradioactive molecules, for example biotin. The probe and the target chromosomes or nucleic acids are denatured. Complementary sequences in the probe and target are then allowed to anneal. After washing and incubation in fluorescently labelled affinity reagents, a discrete fluorescent signal is visible at the site of probe hybridisation FISH has several applications both in research and diagnosis areas.
In this presentation, briefly, the way to make FISH probes by employing chromosome microsurgery will be explained and some of the applications of FISH probes will be mentioned.

P0337. Genetic counselling of the families of t(1;11)(p36.22;q12.2) and t(12;14)(q15;q13) carriers with breakpoint mapping at the BAC clone resolution

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. Empirical data of 60 pregnancies from 10 families of t(1;11)(p36.22;q12.2) of one pedigree and empirical data of 11 pregnancies from 3 families of t(12;14)(q15;q13) of a second pedigree were collected. Probability rates for the occurrence of an unfavorable pregnancy outcome were calculated according to the method of Stengel-Rutkowski and Stene. Chromosome investigations were done by combining at least two different banding techniques, fluorescence *in situ* hybridization (FISH) with probes corresponding to 1p36 and 11q12 and M-FISH.. Breakpoint locations and the 1p36 and 11q12 translocation fragment sizes were estimated. The chromosome 1 breakpoint is located between BAC clones AL096841 and AP001098 (1p36.22) whereas the chromosome 11 breakpoint is located at AP003721 (11q12.2). Any other rearrangements and changes of chromosome 1 were not found by CGH method. M-FISH investigation of family 2 confirmed the breakpoint position obtained by banding techniques. Based on indirect analysis of pedigree one it was found, that probability rate for the birth of a child with unbalanced karyotype (monosomy 1p36.22→pter together with trisomy 11q12.2→qter after 2:2 disjunction and adjacent 1 segregation) is <0.9% (0/50) (low risk), for a stillbirth 2 .0±1.9 % (1/50) and for miscarriages 34±6.7% (17/50) For pedigree two there was found no risk for an unbalanced progeny at birth, and low risk for miscarriages of about 1% (1/11) after ascertainment correction. The data were offered for genetic counselling of the families (*BMBF project POL 03/025 and DPJ 05/005 KBN Polish-German project no 5253, KBN Polish Project No 106 078)*

P0338. Analyses of complex Philadelphia chromosome translocations in chronic myeloid leukemia by conventional and moecular cytogenetics

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Chronic Myeloid Leukaemia is a clonal haematological malignancy, characterised by overproduction of granulocytes and accounts for approximately one quarter of all leukaemia cases. In the majority of patients there is replacement of normal bone marrow by cells with an abnormal chromosome - the Philadelphia (Ph) chromosome due to the translocation between chromosomes 9 and 22.
Routine diagnostic methods for detection of the bcr-abl translocation

include conventional cytogenetics, reverse transcriptase-polymerase chain reaction (RT-PCR), and more recently, fluorescence in situ hybridization (FISH) analysis. Routine cytogenetics is sensitive at the initial diagnosis and during treatment.
Bone marrow samples from 50 patients with chronic myeloid leukemia (CML) were investigated using cytogenetic methods. Fluorescent in situ hybridization (FISH) with BCR-ABL probe was used to confirm and/or complete the findings.
Five variant Philadelphia chromosome (Ph) translocations were identified. Three-way Ph translocations were found in four patients.
FISH analyses allowed more accurate characterization of these complex translocations with subtle abnormalities and the identification of cryptic rearrangements.

P0339. Dihydropyrimidine Dehydrogenase gene *(DPYD)* encompasses the common fragile site, *FRA1E.*

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Common fragile sites represent components of normal chromosome structure that are particularly prone to breakage under replication stress. Although the cytogenetic location of 88 common fragile sites are listed in the Human Genome Database, the DNA at only fourteen of them has been defined and characterized at the molecular level. We identified the precise genomic position of the common fragile site *FRA1E*, mapped to the chromosomal band 1p21.2, and characterize the genetic complexity of the fragile DNA sequence. *FRA1E* extends for 370 kb of genomic region within the dihydropyrimidine dehydrogenase (*DPYD*) gene, which genomically spans approximately 840 kb. The 185 kb region of highest fragility, which is account for 86% of all observed breaks at *FRA1E*, encompasses the central part of *DPYD* including exons 13 to 16. *DPYD,* encodes dihydropyrimidine dehydrogenase (DPD), which is the first and rate-limiting enzyme in a three-step metabolic pathway, involved in degradation of the pyrimidine bases uracil and thymine. Deficiency in human DPD is associated with autosomal recessive disease, thymine-uraciluria, and with severe 5-fluorouracil toxicity in cancer patients.

P0340. Complex chromosomal rearrangements in ten patients with reproductive problem

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Complex chromosomal rearrangements(CCR) are rare aberrations in phenotypically normal persons. The rearrangements involving three or more chromosomes are usually associated with reproductive impairments as infertility or repeated spontaneous abortions. We found 10 cases with balanced CCRs in 2,907 patients with reproductive problem through cytogenetic investigations. The most common CCRs with simple unidirectional rearrangements with one break per chromosome were found in 4 males and a female. The simplest CCRs with two or three simultaneous independent simple reciprocal translocations were 3 females. A female carried a CCR with a simple reciprocal translocation and a three-way exchange simultaneously. The complicated CCR involving more breakpoints than chromosomes observed in a female referred for repeated spontaneous abortions.

P0341. Prenatal diagnosis of an unusual transmission of a complex chromosome rearrangement

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The occurrence of complex chromosome rearrangements is extremely rare. The majority of balanced complex translocations (involving three or more chromosomes) have been reported in women ascertained through miscarriages or the birth of an abnormal child. Men are ascertained because of primary infertility. Here we report on a foetus with an unusual transmission of a complex chromosome rearrangement. A 28-years-old woman was referred for chromosome analysis on chorionic villi because of an increased nuchal translucency detected using foetal ultrasound examination at 12 weeks' gestation. The karyotype showed a derivative chromosome 18 and a pericentric inversion of chromosome 3. Father's karyotype revealed a translocation between 3 chromosomes (3,8 and18) with an additional pericentric inversion in the translocated chromosome 3 : 46,XY,inv(3)(p12q22)t(3;8;18)(p22;p22;p11.2). Fluorescence in situ hybridization (FISH) was performed using chromosome paints and subtelomeric probes to confirm the involved chromosomes and to localize the breakpoints. The foetus inherited the der(18) chromosome leading to a partial trisomy 8p and a partial monosomy 18p. He also inherited the paternal chromosome 3 with a pericentric inversion but without the translocation. Indeed, a crossing-over occurred outside the inverted segment leading to an entire chromosome 3. The higher risk of producing an abnormal child and the different modes of chromosomes segregation will be discuss.

P0342. Molecular cytogenetic detection of *de novo* cryptic unbalanced chromosomal rearrangements by array CGH and subtelomere FISH in two patients with congenital anomalies and dysmorphic features

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Array based comparative genomic hybridization (a-CGH) and subtelomeric fluorescence *in situ* hybridization (tel-FISH) has proven to be very useful tools in the detection of aneusomy due to cryptic chromosomal rearrangements. We report here *de novo* submicroscopic rearrangements detected by these methods in two patients with congenital malformations and dysmorphic features but normal karyotypes. The first patient with dysmorphic features showed an unbalanced chromosomal rearrangement due to t(7;9)(q36.1;p23) leading to a deletion of 7q tel and duplication of 9p ter, detected by array-CGH and later validated by subtelomere FISH. The second patient with congenital anomalies showed an unbalanced rearrangement due to t(2;20)(q 37.1;20p13) leading to a deletion 2q37-qter and duplication of 20ptel, which was confirmed by subtelomere FISH. To our knowledge, deletion and duplication seen in these two cases are not reported earlier. About 14 cases with deletion of 7q36.1-qter and duplication of other telomeric regions excluding 9p; and 9 cases of deletion 2q37.1-qter and duplication of other telomeric regions excluding 20p, are reported earlier. Clinical presentation of the two patients are discussed. Array-CGH and subtelomeric FISH techniques enable us to establish a better genotype-phenotype correlation and provide with valuable information that are extremely helpful in genetic counseling and medical care of the patients and their families.

P0343. Connexin31 can induce neuronal differentiation via a non-gap junctional mechanism

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Connexins (Cx) are the major protein subunits of gap junctions, aqueous pores in the plasma membrane, which allow passage of molecular messengers, short peptides and ions between cells to provide a mechanism of synchronised cellular response. Around 20 human Cx isoforms have been identified and Cx mutations in the 31 kDa isoform, Cx31, underlie a myriad of human diseases, including dominant or recessive skin disease, dominant or recessive deafness or dominant neuropathy with deafness. We previously demonstrated that Cx31 was expressed in differentiating keratinocytes in skin. Here we show that endogenous Cx31 is also expressed in human neuronal cell lines, particularly in differentiated neurones. Exogenous Cx31 expression induced neuronal differentiation in the human neuronal cell line SH-SY5Y, but not keratinocyte differentiation in primary human keratinocytes. Neither the autosomal dominant neuropathy with hearing loss mutation (66delD)Cx31 nor the (R42P)Cx31 skin disease associated mutation are able to traffic to the plasma membrane and form functional gap junction channels. Remarkably, the (R42P)Cx31 mutant can nevertheless induce neuronal differentiation to a level equal to wild-type Cx31, whereas differentiation is reduced after (66delD)Cx31 expression. As well as indicating a potential disease mechanism for the neuropathy with hearing loss Cx31 mutation, this work demonstrates a tissue-specific function of Cx31 and provides, for the first time, evidence that Cx proteins are essential to non-junctional aspects of cell biology.

P0344. Cytogenetic study of Fanconi Anemia in Iranian patients: 10 years experience

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Background: Fanconi's Anemia (FA) is the most common genetic form of aplastic anemia which is an autosomal recessive disorder. The features seen in FA are growth retardation, skin hypo-hyper pigmentation, combined radial ray and thumb deformities, hypogenitalism, microcephaly, renal malformation and microphtalmia. Leukemia, carcinoma or liver tumor is the most frequent malignancies seen in the course of the disease.
Aim: To investigate 400 suspected Fanconi's anemia patients referred to the Iranian Blood Transfusion Organization (IBTO) to confirm diagnosis.
Methods: MMC was used to study induced chromosomal breakage in all those 350 patients. Peripheral blood was cultured in the presence of phytohemagglutinin. For each patient two cultures were set up.
Results: In total sixty- one were cytogenetically diagnosed as Fanconi anemia (~%19.2). Most patients had high, spontaneous and induced chromosomal breakages. No relationship was found between the clinical severity of the disease, age of the onset and the anemic state. In our study the percentages of female affected was slightly higher than male (19.7% verses %17.7) which is different from what have been reported by other researchers. We found in 3% patients mosaic situations for FA. They had 2 populations of peripheral T cells: one with the increased chromosomal breakage typical of FA and one with wild-type cytogenetics. The wild-type clone may result from somatic reversion of a mutated FA allele.In summary, although the initial diagnosis of FA is made by the characteristic clinical features but the definitive diagnosis can be achieved by cytogenetic investigation.

P0345. 12 years experience of Cytogenetic Investigation in Iranian Leukaemic Patients

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We analyzed bone marrow and peripheral blood samples of more than 900 Iranian Patients referred from Major hematology-oncology centers at Tehran and provincial capitals. They were either suspected of leukemia at presentation or being monitored for their response to medication. Bone marrow or/and blood cells were cultured, harvested and G-banded according to the standard protocols. Chromosome analysis was performed following ISCN guidelines. The patients were divided into six major groupings as far as the leukemia subtypes were concerned: CML, AML, ALL, MIDS, Lymphoma and others. They were more male patients than female (1.32: 1 ratio). In terms of sample type most cases had bone marrow aspiration whereas peripheral blood was utilized only in fraction of cases. The common typical chromosomal abnormalities as well as rare and complex forms were observed. The overall chromosomal abnormality rate obtained was around 50%. The breakdown figures for different categories were roughly as follows: 80% in CML, 40% in AML, 25% in ALL, 30% in MDS and 50% in other types. Compared to published data, the observed chromosomal abnormality rate in the present study is considered average.

P0346. Cytogenetic Analysis of Infertile Irainian 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 823 blood samples from infertile men which 617 of them were oligospermic and azoospermic. Constitutional chromosome aberrations were diagnosed in 274 of these patients. We have observed 26.4% chromosomal abnormality in azoospermia men, that is compatible with the data from literature.
The following abnormal chromosome complement were found:
46,XX;47XXY;47,XYY;48,XXXY;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)($q_{11.23}$);46,X,inv(Y)($p_{11.2}$;$q_{11.22}$).
We have found some patients with complex structural and aneupoloidy abnormalities:
× 46,XX,inv(9)($p_{11;}q_{13}$)/47,XXY,inv(9)($p_{11;}q_{13}$)[4]
× 47,XXY[93]/48,XXY+mar[4]/48,XXXY[2]
× 47,XXY,inv(9)($p_{11;}q_{13}$)
× 47,XXY,t(1;17)($p_{36.1;}q_{21}$)
× 46,X,del(Y)($q_{11.2}$)[98]/45,X[6]
× 47,XXY,inv(9)($p_{11;}q_{13}$)/t(10;22)($q_{26.3;}q_{13.1}$)
× 46,X,idic(Y)($p_{11.32;}q_{11.32}$)[27]/45,X[36]/46,XY[2]
We believe that many infertilities especially severe oligospermic and azoospermic cases raises the need for a cytogenetic analysis besides molecular techniques to reveal the existence of any genetic abnormalities

P0347. Cytogenetic analysis in 2,907 patients with reproductive problem

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Cytogenetic investigations were performed on 2,907 patients with reproductive problem as primary infertility or recurrent spontaneous abortions. The overall frequency of major chromosomal abnormalities was 12.4%. The frequency of chromosomal abnormalities was seen to be higher in males(14.4%) than in females(9.7%). The frequency of chromosomal abnormalities in the primary infertility group(22.4%) is considerably higher than that found in the recurrent spontaneous abortion group(7.3%). The frequencies of chromosome abnormalities between males(6.5%) and females(8.2%) in the recurrent spontaneous abortion group were not significantly different($p>0.05$). However the rates of chromosome abnormalities between males(24.3%) and females(16.1%) in primary infertility group were significantly different($p<0.05$). Sex chromosome abnormalities were detected in 9.9% of the males and in 2.2% of the females. Reciprocal translocations exhibited the highest frequency of autosomal chromosome aberrations. Robertsonian translocations and inversions were followed. Supernumerary maker chromosomes were found in three cases. Structural imbalanced aberrations of autosomal chromosomes were detected in four cases. High frequency of chromosome abnormalities found in patients with reproductive problem suggested that a chromosome analysis and genetic counseling should be performed on infertility couples before the application of an assisted reproduction technique.

P0348. Interstitial deletion of 13q in a boy with microcephaly, psychomotor retardation and mild dysmorphic features

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Partial deletions of the long arm of chromosome 13 are associated

with a widely variable phenotype. The most common findings are growth retardation, craniofacial dysmorphisms, digital and limb abnormalities, cardiac and brain defects, and mental retardation. The various manifestations depend on the size and location of the deletion, with a critical region for severe malformations being proposed in 13q32 (Brown et al, 1993).
We report on a fifteen month old boy who was referred for karyotyping because of microcephaly, growth and psychomotor retardation, and mild facial dysmorphic features. Conventional chromosome analysis (GTG-banding) revealed an interstitial deletion in the long arm of chromosome 13. With array comparative genomic hybridization (array-CGH) the size of the deletion was estimated to be between 14.9 and 16.1 Mb and the exact karyotype was defined as 46,XY,del(13)(q21.32q31.1). The karyotypes of the parents were normal. An accurate estimation of deletions is important for genotype - phenotype correlations and this case contributes further data to phenotypes associated with rare monosomies.
Brown S, Gersen S, Anyane-Yeboa K, Warburton D. Preliminary definition of a “critical region “ of chromosome 13 in q32; report of 14 cases with 13q deletions and review of the literature. Am J Med Genet. 1993, 45:52-59

P0349. 7p15.3-21.2 deletion: a recognizable phenotype

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Syndactyly may be an isolated trait with autosomal dominant inheritance. In combination with other congenital anomalies or mental retardation, an underlying syndrome should be suspected. We report a 21-years old woman with a 7p deletion. She shows dysmorphic features, is mentally retarded and retarded in growth. Chromosomal analysis showed a 46, XX,del(7)(p15.2p21.2) karyotype. The region that is deleted contains the TWIST-gene, involved in Saethre Chotzen syndrome. Patients with identical breakpoints are rarely reported in the literature. Reported patients with a similar phenotype had different breakpoints (Grebe, Am J Med Gen, 44:18-23 (1992) and Wang, J Med Genet, 30:610-612 (1993)). We reevaluated the breakpoints of our patient. They appeared to be identical to the breakpoints of the patients reported in literature (karyotype: 46,XX,del(7) (p15.3p21.2)). We compare features of the face and hands with similar patients reported so far and follow-up data of our patient from baby to adult are presented.

P0350. Subtelomeric rearrangements screening in 31 patients with devepolmental delay including rare loss of 14q

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Subtil subtelomeric chromosome rearrangements in children with unexplained combination of developmental delay/mental retardation with dysmorphism and congenital anomalies have been subject of intensive investigation in the past several years. The investigation perforemed so far revealed wide variation in the frequency of subtelomeric aberrations rangin from 0-35%, with an average detection rate of about 5%. In this study we performed the screening for subtelomeric chromosome rearrangements with multicolour FISH assay in order to determine the frequency of aberrations in our group of children with developmental disabilities and contribute to our knowledge on the clinical significance of subtelomeric rearrangements. This investigation included 31 children with developmental delay, dysmorphic features and / or congenital anomalies, and normal karyotype. The analysis was performed using slides obtained by short-term culture of peripheral blood lymphocytes and multi-colour FISH probe panel ToTelVysion (Vysis). Aberrations of subtelomers were detected in 2 (6.4%) of patients including rare observation of loss of 14q subtelomeric region. Our patient is 5-years old girl with microcephaly, dysmorphic features including high forehead with bitemporal narrowing, epicantus, broad nasal bridge, hypoplastic nares, dysplastic ears, high arched palate, small capred-shaped mouth and receeding chin. She showed mild developmental delay, but detailed clinical and laboratory investigation did not show additional abnormalities. Results of this investigation point out the usefulness of subtelomeric screening and present evidence that a continual cytogenetic analysis facilitates detection of rare and new subtelomeric rearrangements as well as a better understanding of their role in the development of children.

P0351. Strategies for detection of chromosome abnormalities in children with developmental delay.

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Clinical geneticists are faced with an array of choices for genetic testing of children with developmental delay or mental retardation. Without specific features to suggest a particular single gene disorder, most geneticists will pursue chromosome analysis followed by subtelomere FISH testing and/or array-CGH testing if the chromosome results are normal. Because subtelomere FISH and array-CGH testing can show abnormal results for large chromosome rearrangements as well as for cytogenetically cryptic rearrangements, the question has been raised whether it might be effective to start with subtelomere FISH or array-CGH studies and then pursue chromosome analysis if needed. In order to find out what proportion of chromosome abnormalities detected by traditional G-banding could be detected by these alternative tests, five years of blood chromosome data from the Genzyme Genetics Santa Fe laboratory were reviewed. Abnormal chromosome results were reviewed and classified as theoretically detectable or undetectable by a subtelomere FISH panel or array-CGH. Discussion of these results will be presented and the efficacy of different testing strategies will be outlined.

P0352. Preimplantation genetic diagnosis for Duchenne muscular dystrophy (DMD) by fluorescence in situ hybridization (FISH): a case report

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Duchenne muscular dystrophy (DMD) is a lethal X-linked recessive disorder with an incidence of approximately 1 in 3500 males, caused by mutation in the DMD gene, located on Xp21.2. About 2/3 of DMD cases are caused by gross DMD gene deletion mutations.Mutations in the DMD gene result in a progressive muscle degeneration and early death.
We reported a case of a family with a occurence of DMD. By means of PCR deletion of exon 45-50 was founded at one year son with clinical proved DMD. His healthy mother was detected as carrier for this mutation. The preimplantation genetic diagnosis (PGD) was used for detection of deletion in dystrophine gene at next pregnancy.
PGD is a principally new approach for the prevention of genetic disorders, which allows the selection of unaffected IVF embryos for establishing pregnancies in couples. PGD can be applied for monogenic disorders or chromozomal abnormalities using diagnostic protocols based on the PCR or fluorescence in situ hybridization.
Seven embryos were biopsied and four of them were analyzed by FISH method by using exon 46-47 DNA probe. Of these, two affected male embryos , one affected female embryo and one unaffected male embryo were detected, it was transferred.
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P0353. DNA methylation patterns of metaphase chromosomes in human preimplantation embryos

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DNA methylation is an epigenetic phenomenon which is known to have a crucial role in mammalian genome reprogramming, especially during preimplantation development. DNA methylation patterns were studied in 27 IVF human triploid embryos at different stages of preimplantation development (from zygote to blastocyst stage). 5-methylcytosine-abundant DNA segments in metaphase chromosomes were identified with commercially available monoclonal antibodies. 1-3 metaphase

plates from each embryo were analyzed. The pattern of chromosome methylation in early preimplantation embryos and up to blastocyst stage differed essentially from this one in chromosomes of adult PHA-stimulated lymphocytes (Pendina et al., 2005). Undermethylation, non-equal methylation status of homologous chromosomes, hemimethylation and hypomethylation of one or two homologues from triad and sister chromatid exchanges were typical features of methylation pattern in early embryos. Hemimethylated chromosomes appeared to increase in number during cell cleavages. They were few at 2-cell stage and dominated at 8-cell stage. Hypomethylated chromosomes, initially detected in zygote, were typical up to the blastocyst stage. Non-equal methylation status of homologous chromosomes in triads was observed in all metaphases studied. Our results suggest that both active and passive demethylation take place and that maternal and paternal genomes have different reprogramming timing in development of human triploid embryo before implantation. Further research of stage- and chromosome-specificity of methylation pattern in early human embryos is in progress.
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P0354. High frequency of *RB1* aberrant methylation in spontaneous abortions with chromosomal mosaicism

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Aneuploidy screening in PGD programs as well as molecular cytogenetic investigation of spontaneous abortions have revealed a high frequency of chromosomal mosaicism during early stages of embryo development. Molecular basis of chromosome non-disjunction in somatic embryo cells are poorly investigated. But one of the most provocative factors is the disturbance of the cell cycle checkpoints. Recently the significance of promoter DNA methylation as epigenetic mechanism of the cell cycle genes silencing was shown. It is possible that aberration of the embryo genome reprogramming can result in epigenetic inactivation of the cell cycle checkpoints and chromosomal mosaicism. To test this hypothesis we have examined promoter hypermethylation of the *RB1* gene in the first-trimester spontaneous abortions with mosaic chromosomal aneuploidies. The mosaic state of chromosomal abnormality was confirmed by interphase FISH analysis of non-cultured placental tissues with centromere-specific DNA probes. Aberrant promoter methylation of the *RB1* gene in the cytotrophoblast and extraembryonic mesoderm was detected in 5 of 9 (55%) and in 7 of 8 (88%) mosaic abortions, respectively. No cases of abnormal DNA methylation were found in placental tissues of 23 first-trimester induced abortions. Our preliminary results indicate the possible link between epigenetic regulation of the cell cycle checkpoints and genesis of chromosomal mosaicism during early stages of embryo development.
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P0355. Patient with double partial monosomies 21q22.3 and 22pter->q11.2: questions of karyotype-phenotype correlation

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Chromosomal abnormalities involving double partial monosomies are very rare. We report on a mildly dysmorphic boy with congenital heart malformation (ultrasonography) who was examined cytogenetically for del22q11.2. Proband (G1, complicated by pyelonephritis, colpitis; P1, at term, unremarkable) was born with BW=3050g; BL=50cm; OFC=34cm. Muscular hypotonia, decreased reflexes, strabismus, upslanting palpebral fissures, short nose, flat bridge, long philtrum, micrognatia, heart murmur, cryptorchism were present at birth.
High resolution GTG-banding analysis revealed an unbalanced translocation with a breakpoint in 22q11.2 containing the DiGeorge/Velocardiofacial syndrome (DG/VCFS) critical region. Karyotype of the patient was 45,XY,der(21)t(21;22)(q22.3;q11.2),-22 de novo.
FISH using LSI 21 (q22.13-q22.2) and N25 LSI probes (Vysis) was done to characterize the derivative chromosome and to elucidate the DG/VCFS deletion. The translocation breakpoints were distal to 21q22.2 and distal to the D22S75 locus on chromosome 22. Finally double partial monosomies 21q22.3->qter and 22pter->q11.2 were shown.
Normal growth, microcephaly, motor development delay, retinopathy, hypocalcemia, anemia, and immune deficiency were found by follow up examinations. At the age of 5 months hemophagocytic lymphohistiocytosis was suspected which is not characteristic for both monosomies. Pneumonia, hepatomegalia, ascites, acute polyorganic insufficiency developed, and the child dead at age 5.5 months. Multiple heart abnormalities including a secundum type ASD, AP, and a common isthmus of coronary arteries, were confirmed at autopsy, as was thymus hypoplasia (w=4g).
Our patient demonstrated some features of del22q11.2 syndrome (heart malformations, hypocalcemia, immune deficiency, early death) and lack of signs of holoprosencephaly, which has been associated with rare deletions of 21q22.3.

P0356. FISH and PCR Analysis in Two Patients with Ambiguous Genitalia and Double Ring Y.

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We reported on two male cases who presented with ambiguous genitalia and double ring Y chromosome.
We aim to correlate genotype and phenotype of the patients.
We performed GTG banding, FISH using whole chromosome painting probe (WCP), specific locus identifier (LSI) for SRY gene, telomere Xp/Yp probe and X and Y cocktail centromeric probe. Molecular analysis using sequence tagged sites (STS), one located within the SRY gene, and 10 STS on Yq spread over intervals 5 and 6.
Both cases had 3 cell lines, 45,X / 46,X,ring /47,X,+2rings . FISH analysis revealed that rings derived entirely from the Y chromosome ,different in size, some were dicentric, different in genetic materials as only one ring had the SRY gene, case 1 had deleted Yp telomere while case 2 had it intact . Molecular analysis confirmed the presence of SRY gene in both . Case.1 had absence of amplified fragment of SY254, 255, 283, 158, and 160 suggesting the breakpoint was within 6D region. Case.2 had absence of amplified fragment 160 which suggested the breakpoint lies between 6D and 6F. Both cases had ambiguous genetalia , no dysmorphic features and no Turner stigmata. Case.1 had short stature.
Both had the same chromosomal anomalies but at the molecular level they had different break sites. Breakpoints demonstrated that both had no risk of gonadoplastoma , case.1 is a candidate of azoospermia in the future, short stature in case 1 may be explained by deletion of the stature gene near the subtelomeric region of Yp.

P0357. Experience of usage of different DNA-probes in clinical cytogenetics

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Fluorescence *in situ* hybridization (FISH) with different DNA-probes has provided more ability to identify chromosome segments, to reveal cryptic abnormalities that are undetectable using standard banding techniques and to perform chromosomal enumeration.
In our study FISH was performed for diagnosis of structural chromosome rearrangements, marker chromosomes, low-level gonosomal mosaicism and more commonly aneuploidies in prenatal diagnosis.
FISH testing was done on metaphases using WCP8, WCP11,CEPX, CEPY DNA- probes (Vysis) and on nuclei using biotinilated DXZ1 (Oncor), DYZ1 (Lau et al.) DNA-probes and AneuVysion kit (Vysis) under the manufacturer's protocols. Usage of WCP11 DNA-probe allowed to determine derivate chromosome 15 undetectable by conventional cytogenetic method in translocation (11;15)(q24;26.3). In a case with previously detected translocation involving chromosome 11, the breakpoints were altered. Use of the WCP8 DNA-probe allowed to exclude a translocation in case with add (8)(p23). Origin of marker chromosomes was estimated - ish der(Y)(DYZ3+)
and ish r(X)(DXZ1+). Among 33 patients with reproductive problems low-level gonosomal mosaicism in 36,5% was detected; in 51,5% this was excluded; and in 12% mosaicism was confirmed. In 46 (3,4%) from 1362 cases of prenatal diagnosis after CVS, the AneuVysion kit was applied, because of bad quality or small numbers of metaphases. There were no false-negative or false-positive results. Usage of this

DNA-probes does not replace conventional prenatal karyotyping. However it allows to rapidly exclude or to diagnose the more common aneuploidies (trisomies 13,18,21 and monosomy X) and thereby to decrease levels of anxiety for pregnant women. FISH with high-specificity DNA-probes increases the quality of cytogenetic diagnosis and allows to base further recommendations for genetic counselling.

P0358. Atypical cases of Down syndrome in Armenian registry of chromosomal abnormalities

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Among 540 children with birth defects were revealed 58 with Down syndrome (DS). It has been found 4 atypical cases of DS: 46,XY,der (14;21)(q10;q10),+21,mat, 46,XY,der(21;21)(q10;q10),+21,de novo (2 cases). The latter case was accompanied with the balanced reciprocal translocation t(1;16)(p36;q13) without any additional phenotypical effect to supplement DS.

The 4-th case was a 15 days old infant, who was delivered to a 40 years old woman at 36 weeks of gestation. He was referred for cytogenetic study because of the following features: microcephaly, brachycephaly, backward-sloping forehead, prominent occiput and dropped eyelids, hypertelorism, epicantus, short and flat nasal bridge, downturned lips, short neck with a low hairline, spread abdomen distension with hydropic anterior wall, low set umbilicus with uretro-umbilical fistula, ascites, inguinal and umbilical hernias. The infant had low motor activity and died at age of 21 days. Pathological study revealed the massive hemorrhages and oedema of the lung parenchyma, numerous development defects of central nervous system, internal hydrocephaly and congenital heart disease. Conventional cytogenetic analysis using GTG-banding revealed 48,XY,+21,+mar karyotype in all the metaphases scored. The marker chromosome's size corresponds to the 21, 22 and Y chromosomes. It has a centromere with two dark bands in both p and q arms and looks like i(21). FISH analysis was performed, using centromeric 13/21 and Y probes. No additional signal was detected in more than 30 cells. This DS case with a rare cytogenetic abnormality as a full double aneuploidy of autosomes demonstrated typical DS manifestation along with additional dysmorphic features.

P0359. Second trimester screening for Down syndrome and trisomy 18 using biochemical markers present in serum samples of pregnant women in north - west of Iran

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The main objective of the present study was to evaluate the efficacy of second trimester screening for Down syndrome and trisomy 18 in the population of pregnant women in north - west of Iran.

The study involved 300 women who attended screening between 15th and 18th weeks of pregnancy. A blood sample were obtained to measure alpha feto protein (AFP), intact human chorionic gonadotropin (hCG) and unconjugated estriol 3 (UE3). The combined risk (maternal age, AFP, hCG and UE3) was calculated by a computerized risk figure program using a fixed 5 percent false positive rate. The screen positive pregnancies were referred to prenatal diagnosis by standard and molecular cytogenetic methods.

Twenty one screen positive pregnancies for Down syndrome (7%) and 9 screen positive pregnancies for trisomy 18 (3%) were detcted. The mean age in study population was 25.11±5.47, in screen positive pregnancies for Down syndrome 30.62±7.88, in screen positive prengnancies for Edward syndrome 30±4.26 and in screen negative pregnancies 24.75±5.45. Prenatal diagnosis using interphase FISH and standard cytogenetic techniques revealed Down syndrome in 3 screen positive pregnancies. Two screen positive pregnancies for Down syndrome were terminated due to fetal death. Non of the screen positive pregnancies for trisomy 18 were affected.

The results are comparable to those of similar studies except for median values of biochemical markers which demonstrate a significant difference from those reported for other ethnic groups, and can be used for prenatal screening of pregnancies for Down syndrome and trisomy 18 in north - west of Iran.

P0360. A report of a dysmorphic child with an apparently balanced translocation

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Here we describe phenotypic description of a male child referred to our centre. The patient presented with dysmorphic face, congenital respiratory problem defect and abnormalities in feet and hands. Chromosomal analysis was done on preparation made from peripheral blood using standard protocol. G-banding revealed an apparently balanced reciprocal translocation between chromosome 1 and 16.

The karyotype was ascertained as: [46,XY,t(1;16)(p11.2;p12)]. The father had normal karyotype. The mother had an apparently same balanced translocation. The abnormalities present in this child could be caused by the possible deletion of some of the important genes located on regions involved in the breakpoints or it can be just a coincidence.

P0361. Chromosome 8p23.1 euchromatic variant in a mentally retarded boy and his father

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Chromosome 8p23.1 duplications are recurrent rearrangements described in association with developmental delay and congenital heart defect resulting presumably from a gene dosage effect of the *GATA4* gene. Cytogenetically indistinguishable rearrangements transmitted over several generations and without phenotypic consequences have been described. This makes the clinical significance of these duplications unclear, and may lead to hazardous interpretations especially during prenatal diagnosis. Recently the underlying molecular mechanism of these benign inherited forms of cytogenetically visible duplications have been elucidated. High copy number variants of at least two *defensin* antimicrobial gene clusters, embedded within several low-copy-repeat (LCR) clusters, are responsible for this euchromatic variant. These LCR are either region specific or repeated throughout the genome. This complex genomic structure contributes to the highly recombinogenic potential of the 8p23 region surrounded by REPP and REPD 8p chromosome duplicons.

Here we present a 4-year old boy referred to us for hyperinsulinism, speech delay and overgrowth, and his phenotypically normal father, both carrying a cytogenetically visible duplication of 8p23.1. FISH analysis using BAC clones did not show a duplication of the region comprised between REPP and REPD. Molecular analysis of the *α-* and *ß-defensin* gene cluster polymorphisms will be presented. Advantages of real-time quantitative PCR in association with FISH in elucidating the cytogenetic 8p23.1 duplications will be discussed. This observation should contribute to define a general strategy using cytogenetic and molecular tools for the exploration of this complex region.

P0362. Chromosome Breakage Findings in Children with Bone Marrow Failure Syndromes - single centre experience

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Fanconi anemia (FA) is described as an autosomal recessive disorder, characterized by the development of progressive pancytopenia, different congenital anomalies and increased predisposition to malignancy. Nature of cause and therapy management are the main terms that distinguish FA from acquired aplastic anemia (AA).

Specific cellular sensitivity of FA cells to the clastogenic effect of DNA cross-linking agents, such as diepoxybutane (DEB), was used to facilitate the diagnosis of FA among AA patients.

Since February 2004 until December 2005, 18 children with AA and other bone marrow failure syndromes were diagnosed and treated at the Mother and Child Health Care Institute of Serbia "Dr Vukan Cupic" in Belgrade. Chromosome breakage study was performed on

spontaneous and DEB-induced 72 hours cultures of peripheral blood. DEB (0.1µg/ml) was added after 48 hours of culturing and metaphases were examined for chromosomal instability and abnormalities.
In examined group of 18 patients, five of them (27.8%) were found to have increased DEB-induced chromosome breaks. The range of DEB-induced chromosome breaks were: for DEB-insensitive patients 0.00-0.20 and for DEB-sensitive patients 0.58-2.15 breaks per cell. No overlap between DEB-sensitive and DEB-insensitive group was found. The range of spontaneous breaks for DEB-insensitive and DEB-sensitive patients were overlapping: 0.00-0.07 and 0.00-0.27 breaks per cell respectively.
Authors will discuss the significance of DEB test in differential diagnosis of AA.

P0363. Automated microscopy of amniotic fluid cells: Detection of FISH signals using the fastFISH™ imaging system.

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FISH is a valuable adjunct to Cytogenetics, providing a rapid screen for common abnormalities. However, FISH is expensive, labor intensive, and requires a high skill level and subjective signal interpretation. Ikonisys *fast*FISH(TM) is a fully robotic fluorescence microscopy platform requiring only that slide-containing cassettes are loaded into the instrument. The system scans each slide and returns it to the cassette with no requirement for user input. Images of cells are digitally captured and automatically analyzed for the presence of specific FISH signals, that are enumerated and reported. Following scanning, the workstation provides a gallery for each slide, displaying the image of each cell and its FISH signals, so that the operator can confirm the diagnostic relevance of the data reported. We blindly compared Ikonisys *fast*FISH(TM) against manual FISH analysis for 62 amniocentesis samples probed for chromosomes 13, 18, 21, X, and Y. Two pairs of slides were produced from each sample, one pair evaluated using standard manual microscopy and the other using the Ikoniscope(TM) *fast*FISH(TM) Imaging System. 100% concordance was observed between the results obtained using manual microscopy and the automated system. 10 samples were then analysed using *fast*FISH(TM) Auto, that produces a test outcome based on fully automated FISH dot-count analysis. These also demonstrated 100% concordance with manual analysis. This suggests that the automated system is capable of providing accurate detection and quantitation of FISH signals and has potential where the reliability and speed offered by an automated system would be of benefit.

P0364. Molecular-cytogenetic diagnosis of patients with Turner syndrome

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The identification of marker chromosome is important for the patients with karyotype 45,X/46,X+mar. In the dysgenic gonads the gonadoblastoma locus on the Y chromosome (GBY) can be oncogenic. Thus, the presence of Y chromosome material in the karyotype of these patients may cause the development of gonadoblastoma. Gonadectomy is generally recommended for these patients. With the methods of conventional cytogenetics the following karyotypes were revealed at 6 patients with delay in physical and sexual development: 45,X[50]/46,X,del(X)(q12)[50]; 46,X,delY(q11)[60]/45,X[40]; 46,X,del(X)(q12)[60]/45,X[40]; 46,X,del(X)(q12)[72]/45,X[28]; 45,X[70]/46,X,r(X)(p11q21)[30]; 46,X,del(X)(p11.2)[70]/45,X[30]. Cytogenetic diagnoses have been verified by FISH (fluorescence *in situ* hybridization) , using CEPX, CEPY (sat. III), CEPY (alpha sat.) and SubtelXpXq (Vysis, USA; Cytocell Technologies, U.K.) DNA-probes. The investigations were carried out with consultations of Institute of Medical Genetics (University of Zurich). As a result of molecular-cytogenetic investigation in the 1st and 2nd cases we revealed the karyotype 45,X [50]/46,X. ish inv dupY(q11)[50] and 46,X. ish inv dupYp(q11)[60]/45,X[40] correspondingly. In the 3rd case cytogenetic diagnosis was specified as 46,X. ish r(X)(p11q21)(DXZ1+,DXYS129-)[60]/45,X[40]. In the 4th case cytogenetic diagnosis has been turned out to be 45,X[28]/46,XY [72]. In 5th and 6th cases cytogenetic diagnoses were confirmed by FISH analysis. The obtained results demonstrate the importance of FISH for the improvement of conventional cytogenetic diagnosis and further correct and timely medical care for the patients with Turner syndrome.

P0365. Parental-origin-determination-FISH (pod-FISH): a new approach to distinguish homologues chromosomes

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The differentiation of homologues chromosomes (chr) and their parental origin can presently be determined only by molecular genetic methods like microsatellite- or SNP-analysis. Only in exceptional cases a distinction on chromosomal level is possible - e.g. due to variations within the heterochromatic regions of chrs. 1, 9, 16 and Y or the p-arms of the acrocentric chrs. In the absence of such polymorphisms a distinction of the homologues chrs on a single cell level was not possible until now. A recently detected polymorphism called LCV or CNP include polymorphisms up to several Mb in size (Iafrate et al., 2004, Sebat et al., 2004). Due to the big size of the described polymorphic regions we started to develop a FISH-based approach for an inter-individual differentiation of the homologues chrs inherited from different individuals, called parental-origin-determination-FISH (pod-FISH) technique. pod-FISH probe sets are at present chr-specific and are composed of BAC clones covering in summary 122 regions distributed over the whole human genome. One- up to 5-color pod-FISH probe sets were created, evaluated, optimised and verified on a known heterochromatic polymorphism of chr 16. Moreover, a case with a known UPD 15 was used to show the suitability and reproducibility of the method. This new method will open new doors for diagnostic and scientific fields that could not be questioned by now: eg introduction of new diagnostic markers for leukaemia after transplantation. Supported by a grant from the university of Jena, Deutsche Krebshilfe (70-3125-Li1) and IZKF / TMWFK (TP 3.7 / B307-04004).

P0366. Localisation and characterisation of common fragile sites

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Fragile sites (FS) are chromosomal regions of high genetic instability, defined as non-randomly distributed regions that show breaks, gaps, or rearrangements in metaphase chromosomes. These genetic alterations can be shown experimentally after culturing cells under conditions of delayed DNA replication or repair. At least 120 chromosomal loci have been identified as FS in the human genome [http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi].
On the basis of their frequency within the population FS are divided into two major groups, rare and common. Both groups can be further divided according to their chemistry of induction. Rare FS are observed in less than 5% of humans and most are induced by folic acid deficiency, whereas common FS are found in all individuals and the majority (76/88) is induced by aphidicolin, the remaining by bromodeoxyuridine or 5-azacytidine.
Untill now 14 common FS, all belonging to the aphidicolin induced group and expressed with the highest frequency, have been cloned and characterised at the molecular level. The majority of them have been involved in somatic rearrangements found in the chromosomes of cancer cells.
However, the localisation of the bromodeoxyuridine or 5-azacytidin induced common FS and the majority of aphidicolin induced common FS which are expressed with lower frequency, is only roughly known through G-banding. To shed light on those unknown common FS, six-colour fluorescence *in situ* hybridisation (FISH) with BAC probes labeled with fluorescence-conjugated nucleotides is used to determine their precise localisation and their molecular characterisation. Additionally their level of expression will be determined in metaphases of different lymphoblastoid cell lines.

P0367. A study of fragile X syndrome in IBTO research center

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Fragile X syndrome is the most common inherted from of mental retardation. The syndrome is associated with a CGG repead expansion in the 5/UTR of the first exone of the fragile mental retadation1(FMR1) gene. This gene maps toXq27.3 and coincides with the cytogenetic fragile site(FRAXA).The present study deal with the prevalence of fragile X syndrome among individuals suspected for fragile X syndrome that referred from various parts of the country to The blood Transfusion Organization reacerch center of Iran From 1999-2005. Results of cytogenetic performed in a group of 247 unrelated individuals (196 males and 51 females)are presented. In total 32 positive cases were detected. Other chromosomal abnormalities were also found in 15 cases. All cytogenetically diagnosed fragile X patients had mental retardation, attention deficits, hyperactivity disorders, and poor visual contact. Futhermore, we found a positive correlation between the frequency of fra(X) and the clinical characteristics. We emphasize the importance of the clinical evaluation in the study of familial mental retardation, molecular analysis at least for the cases cytogenetically found negative for fragile X, and in the screening of isolated cases with suspect of having the fragile X syndrome.

P0368. Three Cases With Gonosomal Chromosomal Anomalies

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In this study, Cytogenetic analyses were carried out for two cases with primary amenorea and one case with premature ovarian failure diagnosed clinicly at the Gynecology and Obstetrics Department.

Case 1 and 2: Two cases with primary amenorea in 20 and 14 year old women are studied. In ultrasonography, neither uterus nor cervix were found and the vagina was atrophic in both patients, and bilateral solid structures were observed in Case 1. Pathologial investigation of these solid structures has shown that their tissue type is compatible with testis tissue. Blood hormonal levels for Testosterone were measured extremely higher in Case 1 as well as FSH, and LH. However, in case 2, only a slight increase was observed for these three hormonal levels.

Case 3: The third case had premature ovarian failure at the age of 31 years having an atrophic uterus and ovaries as well as substantial increase in blood FSH level.

Chromosomal analyses were performed using modified “whole blood” and Giemsa-Trypsin-Leishman banding methods in our laboratory. For all cases 50 metaphase spreads were investigated. The karyotypes of the first two case were 46,XY and the third one was 46,X,del (X)(q27).

This study also proves the importance and necessity of the cytogenetic investigations for finding out the chromosomal abnormalities as well as for supporting the clinical diagnosis.

P0369. Hairy auricula, atypic facies, omphalocele and congenital heart defect with pericentric inversion 2

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We report a patient who had multiple congenital malformations with pericentricentric inversion of chromosome 2. Chromosomal analysis of the consanguineous parents who presented with a reproductive history of four recurrent spontaneous abortions and two neonatal deaths, showed the presence of the same pericentric inversion of chromosome 2; 46,XX,inv(2)(p11q21) in the female partner.

The physical examination of the case revealed frontal bossing, broad-depressed nasal bridge, abnormal hairy ears, long philtrum, high-arched palate and micro-retrognathia. Beside the several clinical findings of our patient, who shared similar abnormalities which were reported in the four cases of medical literature with pericentric inversion 2 has some distinct and interesting phenotypic appearances such as hairy auricula, omphalocele and patent ductus arteriosus. These findings led us for the suspection of other syndromes which might be a separate entity as the consanguinity of the parents was considered.

P0370. Cytogenetic endpoints as indicators of chromosomal damage in hypertensive patients treated with beta-blocker drugs: *In vivo* study before and after antihypertensive therapy.

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Arterial hypertension represent one of the most frequent diagnoses in the population at large in terms of prevalence and incidence.

Among the most used antihypertensive drugs, the group of chemical known as β-bloquers have been found to have markedly beneficial therapeutic value in treating hypertension. As a results of the high volume of these drugs used and suggestions that some members of this chemical class may produce chromic toxicological effects, the regulatory agencies have become concerned over the possible long-term adverse effects of β-bloquers.

Our previous studies was analyzed the beta-blocker atenolol and reported its genotoxic effect *in vivo* after comparing hypertensive patients with an age- and sex-matched control sample. To complete the study and to bias interindividual susceptibilities we are doing a further study where patients act as their own controls.

In this study SCE and MN constitute the genotoxicity biomarkers used. Their frequencies are measured in peripheral blood lymphocytes of hypertensive patients a) before starting the antihypertensive treatment, b) three months after therapy and c) one year after iniciated the pharmacological treatment.

The results obtained show increases in the frequency of SCE three months after therapy, whereas the values of this biomarker before starting the antihypertensive treatment and one year after iniciated are similar. An “adaptative response” to the drug could explain these results.

As regard the MN assay the results indicate a progressive increase in their frequency and these could show that MN assay is sensitive enough to detect the chromosomal damage resulting from that antihypertensive treatment.

P0371. A Case Of Intrachromosomal Insertion On Chromosome 7 Involving Five Breakpoints

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Intrachromosomal insertions are uncommon rearrangements. Cytogenetic delineation of these abnormalities can be difficult. It is highly probable, that these abnormalities have been misinterpreted as inversions. Fluorescence in situ hybridization technique (FISH) by using different probes made it possible to delineate such chromosomal abnormalities.

We report a de novo case of intrachromosomal insertion on chromosome 7. Following uneventful pregnancy of healthy, unconsanginous parents a newborn baby was born with severe congenital malformations (severe growth retardation, trigonocephaly, short neck, dysmorphic face, cleft palate, digital anomalies). The baby died at 41. day of age.

Chromosomal investigation was performed on peripheral blood lymphocytes and GTG banding analyses at 550-600 band level revealed a derivative chromosome 7. Parental chromosomes were normal. FISH technique was applied by using different probes (WCP 7-Cytocell, P&Q dual painting probe-metasystems, Williams dual color probe-Vysis, 7 p&q telomeric probe-Cytocell). According to the FISH and GTG banding findings, the rearrangement was interpreted as intrachromosomal insertion with the breakpoints; 7p21, 7p15.3, 7q11.23, 7q31.2, 7q34

(Karyotype;

46,XY, der(7)(pter-->p21::q34-->q31.2::q21-->p15.3::q11.23-->q31.2::p15.3-->q11.23::q34-->qter)).

This case shows us how useful the FISH results are for the clarifying

of the complex rearrangements. De novo unusual inversions should be investigated by FISH technique especially in cases with multiple congenital malformations.

P0372. Paternal heterozygous inv(8p23.1) predisposing to duplication 8p23.1?

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Rearrangements of the short arm of chromosome 8 manifest as complex inverted duplications and deletions, or as simple duplications or simple deletions. In most instances band 8p23.1 is involved, and the breakpoints are thought to correspond with segmental duplication sites at the proximal and distal 8p23.1 boundaries. Recent reports have shown convincing evidence that there might be a causal relationship between maternally heterozygous inversions in band 8p23.1 and inversion duplication deletions of 8p in the offspring, due to loop formation and meiotic recombination in meisosis I.
We present three male patients with different chromosome 8p aberrations: i.e. an inv dup del(8p), an inv dup(8p) and a dup(8p). The rearrangements were ascertained by chromosome and FISH analyses, and further characterized by micro array CGH analysis. Two copy number transitions (breakpoints at 8p23.1 proximal and 8p23.1 distal) are observed in all three patients, coinciding with the known segmental duplication sites.
We performed FISH analysis on the parents of all patients to see whether we could find a heterozygous maternal inversion in 8p23.1 to support a causal relationship with the observed aberrations in our patients. The mothers of the inv dup del(8p) and the inv dup(8p) patients indeed carried a heterozygous inversion in 8p23.1, whereas the fathers appeared normal. Interestingly, the mother of the dup(8p) patient had a homozygous inversion 8p23.1, whereas the father had a heterozygous one. It is tempting to speculate that in this case the paternal heterozyous inversion mediated the formation of the abnormal chromosome 8.

P0373. Inverted tandem duplication resulting in a functional disomy of chromosome Xq28.

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Inverted tandem duplications are well-known chromosome abnormalities. The most frequent involves the short arm of chromosome 8 and is associated with a 8pter deletion. It has been demonstrated that segmental duplications (low copy repeats) do mediate these rearrangements. Here we report on an inverted Xq28 tandem duplication associated with a terminal Xq28 deletion. The child's clinical features are suggestive of a chromosome Xq28 functional disomy i.e. growth retardation, developmental delay, microcephaly, failure to thrive, pulmonary veins stenosis, recurrent pulmonary infections and micropenis.
Standard cytogenetic and FISH analysis revealed a Xqter deletion associated with a Xq28 duplication. The abnormal X is inherited from the phenotypically normal mother. Cytogenetic studies after BrdU incorporation and measure of incorporation ratios at the androgen receptor locus show extreme skewing of inactivation i.e. the abnormal X is preferentially inactivated.
The presence of inverted homologous low copy repeats on the terminal long arm of chromosome X suggests that they did mediate the rearrangement. To our knowledge this is the first case of chromosome Xq28 functional disomy caused by an inverted duplication.

P0374. Structural Y-chromosomal aberrations: four cases demonstrating the value of FISH

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The Y-chromosome harbours genes for spermatogenesis (DAZ-gene), sex determination (SRY) and growth (SHOX). FISH analysis for these genes may help in the clinical interpretation of karyotypes with constitutional structural Y-chromosome abnormalities.
We present four male patients in which FISH analysis was helpful. The aberrant Y-chromosomes were characterised by FISH using 8 different probes: RP13-76L22 (Yp11.32), c34F5 (Yp11.31), SRY (Yp11.2), pDP105 (Yp11.2), pDP97 (DYZ3), RP11-160O2 (Yq11.22), 63C9 (Yq11.23) and RP11-479B17 (Yqter).
In two males with oligo- or azoospermia, FISH demonstrated absence of DAZ .
A) mos 46,X,der(Y),del(Y)(q11.223)invdup(p11.2-pter)[47]/46,X,idic(Y)(p11.1-q11.223::q11.223-p11.1) [3]. The second line was not found at karyotyping.
B) mos 45,X[50]/ 46,X,idic(Y)(q11.2)[48]/47,X,idic(Y)(q11.2),+idic(Y)(q11.2)[2].
Two cases were detected in amniotic fluid, screened because of maternal age. FISH, performed nine years and 5 month after birth, respectively, demonstrated absence of DAZ and duplication of SHOX in both cases.
C) mos 47,X,der(Y)del(Y)(q11.23)invdup(Y)(p11.2pter),+der(Y)del(Y)(p11.2) del(Y)(q11.22)[24]/46,X,der(Y)del(Y)(q11.23)invdup(Y)(p11.2pter)[6].
D) mos 45,X[23]/46,XY[1]/46,X,idic(Y)(q11.2)[72]/47,X,idic(Y)(q11.2)x2[4].
Demonstrating the absence of DAZ helped explain the impaired spermatogenesis in patients A en B, and predicted a high risk of infertility in patients C en D. The SHOX duplication may have contributed to the observed increased height (three years in front of age at the age of 9 years) in patient C. The phenotype of patient D is normal at the moment.
In conclusion, FISH analysis in patients with structurally abnormal Y chromosomes may help in understanding (and predicting) their associated phenotypes.

P0375. Mosaic status of lymphocytes in men with Klinefelter Syndrome

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Klinefelter syndrome (KFS) is characterized by gynaecomestia and hypergonadotrophic hypogonadism and azoospermia. Recently few studies have shown that KFcases have 46, XY cell line and in such cases there may be isolated foci of spermatogenesis. Such cases have better prognosis on assisted reproductive techniques (ART) .So we tried to look for low-level mosaicism in ten infertile Klinefelter patients who did not show features of hypogonadism to identify for presence of normal cell line which could not be identifyied on analyzing twenty metaphases. In these cases we analyzed 150 well spread G banded metaphases in each case and found that two cases showed 4-5% 46, XY cell line.This had not been identified on analyzing 20 metaphase spreads.
The main problem with conventional karyotyping is that routinely 20 metaphases are analyzed and counted, this may miss low-grade mosaicism. Such KF cases(diagnosed as pure 47,XXY) may have foci of spermatogenesis, which could be used for TESA in ART. Therefore analyzing a large number of metaphases may detect low percentage of 46, XY cell line and such cases carry a good prognosis on ART.
Recent techniques like FISH (florescence in situ hybridization) are rapid and sensitive and can help in analyzing a large number of metaphases and interphases cells in short time and can detect cryptic and low level mosacism, which may have been missed cytogenetically.

P0376. Submicroscopic DER(X) detected by FISH - Unusual finding in a Klinefelter Syndrome patient

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Our presentation deals with Klinefelter syndrome patient (18-years-old male) with unusual FISH finding. Common cytogenetic examination identified abnormal karyotype (47,XXY).
Due to some mild phenotypic features that could result from possible mosaicism FISH examination was indicated. Molecular cytogenetic

analysis was carried out with centromeric and locus specific probes (DXZ1, KAL) to chromosome X and a heterochromatine probe (DYZ1) to chromosome Y. The FISH analysis demonstrated presence of a very small derivative X-chromosome that consist of only centromeric segment. It was present in 55% of cells. This small centric fragment was not identified by a common cytogenetic analysis.
At the beginning of the FISH analysis of mitotic chromosomes (using the X-centromeric probe) the free X-centromeric fragment was considered to be only a fluorescent artefact. Besides this one X chromosome showed only weak hybridisation signal on the centromeric region. The previous FISH result on mitotic chromosomes had therefore the similar appearance as this in mitosis of a normal male. The second X-chromosome with the weak signal on centromeric region was identified by subsequent FISH analysis using the Kallman region specific probe combined with X-centromeric one. It is not excluded that the presence of the small derivative X-chromosome could lead to possible X-chromosome mosaic in other tissues of the patient. The poster discuss possible implications of this result to postnatal and prenatal diagnosis of X-chromosomal aneuploidies. Our work was supported by research project of the Ministry of Health of the Czech Republic No. 00064203.

P0377. Chromosome study for diagnosis and prognostic evaluation of leukemia

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Cytogenetic study has been carried out on 71 individuals diagnosed with some kind of hematological disorder. Conventional and FISH technique were considered in cultured and uncultured bone marrow samples and ~50 cells were examined using software for karyotyping and FISH imaging. Conventional G-banding study has collected information on multiple abnormalities in 65% cases, including t(10;11); del(5q); del(7q); -7; t(15;17); t(3;6); +8; del(1q); -Y; t(X;14) in Ph+ve CML; del(20q); t(4;7); t(14;18); -13; t(9;22)+inv(16); ins(1)+inv(6)+t(7p;9q)+t(8;21); 48,XX,+6,+21/51,XXX,+6,+21(x2),+m; paracentric inv(8); t(9;22;21); 46,XY/45,XY,t(4;14),der(14),inv(4); etc. FISH study was efficient to detect rearrangements in G-banding negative cases. G-banding has identified two cases with three-way translocations, t(9;22;11) and t(9;22;21) where FISH result showed bcr-abl chimerical fusions. The patients detected with multiple rearrangements had poor prognosis. CML patients with three-way translocation showed poor treatment outcome. One CML with 46,XY/45,XY,t(4;14),der(14),inv(4) configuration received bone marrow transplant and responded well. Two AML patients with complex clonal abnormalities expired within 2-3 months after detection. G-banding study has also identified constitutive abnormalities in 6 patients, including pericentric inversion in 9 (2), Y (2) and X (1), and mosaic hermaphroditic karyotype 46,XY/46,XX (1). The study has yet to correlate with antibody markers and treatment outcome. This study indicates the importance of conventional technique for primary diagnosis as well as follow-up programme for recognizing clonal evolutions and multiple rearrangements. However, FISH must be considered as an adjunct to G-banding technique.

P0378. Detection of RB1 deletions by fluorescence in situ hybridization in healthy first degree relatives of patients with lymphoproliferative disease

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INTRODUCTION: Interstitial deletions of 13q14 are found in hematological malignancies.
The RB1locus is within the deletion interval of del (13)(q12q14) in leukemic cells, it is a tumor suppressor gene, responsible for the development of solid tumors. Its role in hematological diseases is unclear.
AIM OF THE STUDY: We showed that RB1gene deletion. is not restricted in pts with lymphoproliferative disorders but it is expanded in 1stdegree relatives.
PATIENTS: FAMILY-A:a 73y old man with HCL.and a son (49y) with B-CLL.Two children (10,12y) are studied.
FAMILY-B:a 69y old man has B-CLL.A brother and two sisters (71,63,65y) have an udefined lymphoproliferative disease with an IgAk paraproteinemia. Six children (28-35y) are studied.
FAMILY-C: two brothers(72,75y) with B-NHLand.two sons (25,45y) are included.
METHODS: Bone marrow specimens and PB lymphocytes were cultured using standard techniques. Thirty GTG banded metaphases were analyzed (ISCN1995).
FISH was performed using the LSI13 probe so(VYSIS).
RESULTS: The karyotypes looked normal. With FISH we isolated single signals of RB1gene in both groups. We evaluated as a deletion the presence of a single signal in 10% of the interphase nuclei (200 analyzed) or in 2 metaphases (20 analyzed).
CONCLUSIONS
1) FISH technique is valuable for detection of RB1 gene deletion in pts with lymphoproliferative disorders and in 1stdegree relatives.
2) The del13q14 is compatible with an inherited condition.
3) The1st degree relatives are in high risk of a forthcoming similar disease.
4) Our results are compatible to that in the literature which are referred as familial
leukemia, lymphoma, multiple myeloma, hairy cell leukemia.

P0379. Report of 2 cases of translocation of chromosome Y and an autosome chromosome in azoospermic males

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Besides sex chromosome numerical aberrations, several structural abnormalities such as translocations, markers or inversions are more frequently found in the karyotype of infertile men (Thielemans *et al.*, 1998 ; Gekas *et al.*, 2001). Y-autosome (Y/A) translocations have been reported in association with male infertility. Different hypotheses have been made as to correlations between Y/A translocations and spermatogenetic disturbances. In an early review by Smith et al, 1979 it was postulated that Y:acrocentric translocations are less often associated with infertily & hypogonadism that are Y;non-acrocentric translocations.
We are reporting 2 cases of balanced translocation of chromosome Y and an autosome in azoospermic males. The first case, a 31 year old male patient with 4 years history of infertility due to azoospermia has 46,X,(Y;5)(p11;q13) and the second case, a 34 year old male with azoospermia has 46,X,(Y;2)(q11.2;q35).
In the Mendelian cytogenetic network database of the 8 cases with breakpoints of Yq11.2, 6 have aoospermia/oligospermia, and the one case with breakpoint at Yp11 also has Azoospermia/Oligospermia. This would suggest that the Y;autosome translocations involving these breakpoints are associated with Azoospermia/Oligospermia or as more generally postulated will lead to meiotic disturbances.

P0380. Male Shrew Recombination Maps for Each Chromosome Identified by DAPI-banding

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Studies in human and mouse meiotic chromosomes demonstrated that the rate of recombination was increased in pretelomeric regions and reduced near the centromeres. An excess of recombination frequency was detected in GC-rich regions. We analysed the chromosome-wide and regional patterns of meiotic recombination in another mammalian species: the European common shrew (*Sorex araneus* L.). This species is characterised by unprecedented chromosome polymorphism and X/Y1,Y2 system of sex determination. We generated the first cytological recombination maps for each autosome of this species identified DAPI-banding. We prepared synaptonemal complex spreads from spermatocytes of male shrews of various karyotypes, identified each autosome by DAPI-banding, validated this identification by fluorescence *in situ* hybridization of chromosome-specific DNA libraries, and mapped recombination along individual chromosome arms, using immunolocalisation of MLH1, a mismatch repair protein that marks sites of crossingover. Majority of bivalents and the autosomal arm

of sex trivalent demonstrated high recombination frequency near telomeres and low frequency near the centromeres. However each bivalent had unique pattern of crossover distribution along its length. This pattern was apparently determined by interplay between chiasma interference and the region specific differences in GC-content. Taking an advantage of clear DAPI-banding revealed in the spreads of shrew spermatocytes we demonstrated that recombination frequency was 1.5 times higher in G-negative regions than in G-positive. We found that the chromosome arms, which had low frequency of recombination in normal karyotype, were prone to pairing failure in complex synaptic configurations, such as chain of IX.

P0381. Tiling resolution array CGH reveals a low percentage mosaic trisomy 8 in a patient with mental retardation

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For nearly two years, we have been using tiling resolution BAC arrays (>32,000 clones) in a diagnostic setting. This novel technique has been implemented in our department in order to unravel a diagnosis in patients that initially showed a normal karyotype, but still are suspected of having (submicroscopic) chromosomal imbalances. Here we report on a 68-year old male individual with mild mental retardation. Previous chromosomal analysis and extensive FISH analyses had revealed a normal male karyotype and normal FISH results. Subsequent array CGH analysis of the DNA of this patient revealed a normal pattern for all chromosomes, except for chromosome 8. Although no discrete gains and/or losses were detected on chromosome 8, all BAC clones from this particular chromosome displayed a positive test over reference ratio instead of the expected scattering around zero. The average ratio of the BAC clones on chromosome 8 was 0.1, indicative for a possible mosaic trisomy 8. We were able to confirm this mosaicism by performing interphase FISH analysis with a centromere probe of chromosome 8. After analysing 400 nuclei, three signals were detected in 29 nuclei, whereas the other 371 nuclei showed the expected 2 signals for chromosome 8. This result is indicative for a ~7 % mosaicism for trisomy 8 in this patient as was initially identified by array CGH.This finding clearly demonstrates the power of tiling resolution BAC arrays in detecting even low grade chromosomal imbalances as a result of low percentage mosaicisms.

P0382. Emerging patterns of cryptic chromosomal imbalances in patients with idiopathic mental retardation and multiple congenital anomalies

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Chromosomal abnormalities are a major cause of mental retardation and multiple congenital anomalies (MCA/MR). Screening for these chromosomal imbalances has mainly been performed by standard karyotyping. Previous array CGH studies on selected patients with chromosomal phenotypes and normal karyotypes suggested an incidence of 10-15% previously unnoticed *de novo* chromosomal imbalances. Here we report on array CGH screening of a series of 140 patients with idiopathic mental retardation and multiple congenital anomalies (MCA/MR) but normal karyotypes. Submicroscopic chromosomal imbalances were detected in 20% (28/140) patients and included 18 deletions, 7 duplications and 3 unbalanced translocations. Seventeen from twenty four imbalances were confirmed *de novo* and 19 were assumed to be causal. Excluding subtelomeric imbalances, our study identified 11 (8%) clinically relevant interstitial submicroscopic imbalances. Taking into consideration this and previously reported studies, array CGH screening with a resolution of at least 1 Mb, has been performed on 432 patients with MCA/MR. Most imbalances are non-recurrent and spread across the genome. In at least 8.8% (38/432) of these patients *de novo* intrachromosomal alterations have been identified. Hence, array CGH should be considered as an essential aspect of the genetic analysis of patients with MCA/MR. In addition, in our study 3 patients were mosaic for a structural chromosome rearrangement. One of these patients had monosomy 7 in as little as 7% of the cells, illustrating that array CGH allows the detection of low grade mosaicisims.

P0383. Investigation of cytogenetic causes of mental retardation in Romanian children. Results of a two years study

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OBJECTIVE: The aim of this study was to investigate chromosomal abnormalities in children with mental retardation (MR).

MATERIAL AND METHOD: 80 children with MR were included in this study over a period of 2 years (2001-2003).

All patients were evaluated, by clinical and paraclinical exams (including dysmorphological features, psychological tests, neuroimagistic studies). Cytogenetic investigation consisted of karyotype examination (GTG banding) and fragile site induction.

RESULTS: The chromosomal studies identified the following abnormalities: fragile X syndrome in 4 cases, trisomy 21 in 4 cases, partial trisomy 18 in one case, deletion 1q42 in one case, 3p duplication in one case. Several cases have been diagnosed with various genetic syndromes: Williams syndrome (one case), Cornelia de Lange syndrome, Berardinelli-Seip syndrome (2 cases), based on the clinical features, but without a molecular genetic confirmation. 18 mentally retarded children with different dysmorphic features, had not revealed any numerical or structural chromosomal abnormalities. Almost all of these patients have severe MR and other neurological or psychiatric disorders.

CONCLUSIONS: Genetic abnormalities are an important cause of mental retardation in children. Cytogenetic analysis should be included in the protocol of investigation in all children with mental retardation.

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P0384. BAC array CGH reveals five genomic aberrations in 30 patients with idiopathic mental retardation

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Array using 2,173 BAC clones covering the whole human genome has been constructed. All clones spotted were confirmed to show a unique signal at the predicted chromosomal location by FISH analysis in our laboratory. A total of 30 individuals with idiopathic mental retardation (MR) were analyzed by comparative genomic hybridization using this array. Three deletions [46XY,del(15)(q11.2q 12)mat, 46,XX,del(1)(1q43qter), and 46XY,del (3)(p21p21)de novo], one duplication [46XY,dup(22)(q11)de novo], and one unbalanced translocation [46XY,der(22) t(19;22)(p13.3;q13.31)pat] could be detected in five patients, which are likely to contribute to MR. The constructed array was shown to be an efficient tool for the detection of pathogenic genomic rearrangements in MR patients as well as copy number polymorphisms (CPNs).

P0385. Apparently balanced t(2;21) „de novo“ with 2q37.3, 21q11.2 and 21q21.1 microdeletions

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Microdeletions of the long arm of chromosome 2 in q37.3 have been detected since 1995 using specific probes and have been associated, in about half the cases, with a phenotype similar to Albright's hereditary osteodystrophy. Few cases have been described with proximal deletions of chromosome 21q, in q11.2 and q21.1, possibly because this region does not include genes responsible for the severe phenotype.

The authors present the clinical description and cytogenetic and molecular findings of a male patient aged 5, referred for cytogenetic studies because of dysmorphic features, mild delay of psychomotor

development with impaired fine motor skills and speech delay. The high resolution GTG banding karyotype revealed an apparently balanced translocation between the end of 2qter and 21q. As the karyotypes of the parents were normal, the child's translocation was *de novo*. Cytogenetic molecular techniques (FISH) were performed on the family and it was concluded that the subtelomeric region on the long arm of chromosome 2 was absent in the child. Because the phenotype of the proband was not characteristic of the 2q37.3 microdeletion, molecular typing of the long arm of chromosome 21 was performed. This showed loss of heterozygosity for markers D21S1911 and D21S11, that is, an interstitial microdeletion involving bands 21q11.2 and 21q21.1.
The authors emphasize the importance of high resolution GTG banding in the characterization of dysmorphic syndromes and of making an accurate clinical description of the patient, comparing the phenotype with those observed in other cases, described in the literature, with similar cytogenetic alterations.

P0386. Clinical features in a girl with a mitotic abnormal segregation of a chromosomal balanced translocation

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We report a 16 years old girl with an imbalanced mitotic segregation of a maternally inherited balanced translocation.
Blood karyotype is 46,XX t(17;22)(p13;q12)mat (8%)/45,XX,-17,-22,der(17) (62%)/47,XX,t(17;22)(p13;q12) +der(22) (30%). Chromosome analysis on other tissues is currently in the pipeline.
Pregnancy and delivery were uneventful. She had congenital hip dislocation and delayed psychomotor development, but no major malformation. Growth was normal. She subsequently showed refractory partial epilepsy, autistic trait, scoliosis, and a neurogenic detrusor dysfunction in the last months.
No similar patients had been described in literature so far, since all the cases reported with a chromosomal mosaicism (Dufke et al, 2001; Kuharya et al, 2002), resulting from a parental balanced translocation, presumably derived their abnormal karyotype from a meiotic error and a postzygotic rescue. Our patient clearly demonstrate that an aberrant mitotic segregation can be a possible althoug rare outcome of a balanced translocation. This should remark the relevance of performing an exhaustive cytogenetic analysis in mentally retarded patients, especially when a chromosomal rearrangement is found within the family.
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P0387. MLPA as a screening of aneuploidy and unbalanced chromosomal rearrangements in spontaneous miscarriages

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Chromosomal anomalies account for no less than 50% of first trimester spontaneous abortions (SAs). Despite establishing the cause of miscarrige is recommended in order to offer an appropriate genetic counselling, cytogenetic study of such specimens entail high rates of culture failure or wrong diagnosis due to maternal cell contamination. Moreover, some authors support the idea that conventional cytogenetics may yield normal karyotypes or selected abnormal ones that allow *in vitro* cell proliferation, suggesting that rates of abnormalities uncommonly seen by classic cytogenetics may be more frequent than the reported ones.
The Multiplex Ligation-dependent Probe Amplification (MLPA) technique permits the detection of copy number changes of subtelomeric DNA sequences for both arms of every chromosome in a single assay. Here we discuss its feasibility (sensitivity, specificity and reproducibility) as a first approach to detect aneuploidy and unbalanced terminal chromosomal rearrangements in spontaneous miscarriages. For this purpose, 182 miscarriage DNA samples were tested with the SALSA P070 and P036B MLPA probe mixes. Results obtained by MLPA were validated by performing targeted DNA microsatellite analysis, karyotyping and/or conventional CGH. As no false-negative results were obtained, MLPA with subtelomeric probes could be employed as a first approach to the screening of aneuploidy in SAs. Nevertheless, the presence of false-positive results suggests the need of revise probes design in order to improve the accuracy of the technique.

P0388. Mosaic variegated aneuploidy

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Mosaic variegated aneuploidy (MVA) is a recessive condition characterized by mosaic aneuploidies, predominantly trisomies and monosomies, involving multiple different chromosomes and tissues. Although it is not typically associated with a distinctive phenotype, some common findings including microcephaly, IUGR have been reported previously in children with MVA. It has been suggested that a non-random gain of chromosomes specific to each somatic tissue might result in higher frequency of certain trisomic cell lines in different tissues. MVA has been previously reported in patients with malignancies, recurrent miscarriages and multiple malformations.
We report a 16- month-old male child born to a non-consanguineous couple , with antenatally diagnosed polyhydramnios and failure to thrive and developmental delay postnatally. In addition he had microcephaly, scaly skin with easy bruising and palmo-plantar atrophy, patchy alopecia, central nuclear cataracts detected by 7 months of age and dysmorphic facial features including flat nasal bridge and short nose, small mouth with high arched palate, crowded teeth, bilateral single palmer crease. His relevant investigations including biochemical studies for peroxisomal dysfunction, skeletal survey etc were normal. However, His karyotype revealed MVA. Out of 93 metaphases studied, hyperdiploidy was found in 17 (47-49 chromosomes) while hypodiploidy in 14 cells. Parental karyotypes were normal.
Here we present a details of cytogenetic analysis of our patient including MVA and its implications to this family.

P0389. Interphase In Situ Hybridization analysis of 14q32 and 13q chromosomal abnormalities in multiple myeloma: About 17 cases

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Multiple myeloma (MM) is a clonal B-cell neoplasia characterized by the accumulation in bone marrow of malignant plasma cells producing a monoclonal immunoglobulin.
Because cytogenetics are often caught out by the low proliferation, and because some chromosomal changes may be cryptic, we have conducted an interphase FISH study anlysing 14q32 and 13q14 rearrangements, which are the most common genetic aberrations in MM, in 17 tunisian patients with MM using a dual-color IGH probe mapping at 14q32 and a probe specific of the D13S319 locus, at 13q14.
Interphase FISH revealed translocations involving the IGH locus in 14 (88.2%) patients.
Eight (47%) patients had 13q14 deletion, seven of whom also had IGH translocation
In conclusion, this study demonstrates the high incidence of the IGH rearrangement in tunisian patient with MM.
Moreover, the D13S319 deletion indicate the presence on this locus of a yet-unidentified gene with a tumor suppressor function.
For a better inderstanding of pathological mechanisms of MM and to assess the incidence of each IGH translocation, other FISH analysis will be further perfomed using probes specific of the most common partner sites of the IGH gene like: Bcl 1 located at 11q13, FGFR3 at 4p16, C-maf at 16q23 and the proto-oncogene c-myc located at 8q24.

P0390. Cytogenetic analysis of pol and gag MSRV sequences in MS patients

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Pathogenesis of MS is still poorly understood. Multiple sclerosis-associated retrovirus (MSRV) is postulated as one of the pathogenic factors of MS.

The aim of our studies was the assessment of MSRV pol and gag copy number in MS patients compared to healthy individuals and persons with myasthenia. FISH studies with biotinylated PCR products of pol and gag sequences were performed on chromosomes, interphase nuclei and chromatin fibers of stimulated peripheral blood lymphocytes.

Although MSRV pol was found in all examined persons, the copy number of this sequence was significantly greater in MS patients (6-24 copies on nuclei) than in myasthenia (4-5 copies) and normal individuals (3-6 copies). In addition, the MSRV pol sequence exists as tandem repeats on chromatin fibers. MSRV pol probe hybridized to chromosomes 1, 2, 3, 4, 5, 7, 10, 14, 17 and X. In the contrary, MSRV gag sequence was found in range 2-4 copies in both MS patients and controls.

In conclusion, evident difference in MSRV pol copy number between MS patients and controls suggests that MSRV pol may play some role in the etiology of multiple sclerosis.

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P0391. A Neocentromere locus at 13q31 in a Supernumerary Marker Resulting in a Mosaic Tetrasomy of Distal 13q

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Neocentromeres are functional centromeres originated in non-centromeric regions of chromosomes. The formation of neocentromeres results in conferring mitotic stability to chromosome fragments that do not contain alpha satellite DNA.

We present a prenatal diagnosis in a 34 year-old gravida, on the 24th week of gestation with ultrasound malformations: large cisterna magna, no renal differentiation, hypotelorism and ventriculomegaly. Cytogenetic analysis of G-banded chromosomes from the amniotic fluid cells and fetal blood revealed a supernumerary marker chromosome in mosaic. Molecular cytogenetics analysis using fluorescence *in situ* hybridization with alpha satellites probes, chromosome specific painting and subtelomeric probes for chromosome 13 was performed as well as comparative genomic hybridization (CGH). These studies showed that this marker was an inverted duplication of the distal portion of chromosome 13q with no detectable alpha satellite DNA. The presence of a functional neocentromere on this marker chromosome was confirmed by immunofluorescence with antibodies to centromere protein-C (CENP-C). The neocentomeric constriction was at band 13q31. Parents decided, after genetic counseling, to terminate pregnancy. An autopsy was performed and the anatomopatologic study revealed a female fetus with facial dysmorphisms, low set ears and renal dysplasia.

Mosaicism, often observed in patients with neocentromeres, suggests that its segregation efficiency is lower than for normal centromeres.

Eleven supernumerary neocentromeric chromosomes originating from the distal region of chromosome 13q have been reported. This frequency suggests this chromosome to have and increased propensity for neocentromere formation. However, there is only one more case described with the location of the neocentromere in band 13q31.

P0392. High-resolution of comparative genomic hybridization improves detection of chromosomal aberrations with prognostic significance in neuroblastoma

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Neuroblastoma (NB) is a genetically very heterogeneous pediatric malignant tumor. The clinical course of NB vary markedly. Therefore molecular and cytogenetic markers are studied as strong predictors of clinical outcome, to amnedclinical staging and aid in treatment planning.

This malignancy is characterized by a broad spectrum of clinical behavior. Low-, intermediate, and high-risk groups have been defined based upon expected outcome following conventional therapy using both clinical and biological criteria. The criteria currently used to assign risk-group are as follows: Clinical stage, MYCN status, Shimada histology and DNA ploidy. Recently, other cytogenetic changes as 3p, 11q deletions and 17q rearrangements may also have prognostic value.

A global view of genetic imbalances in NB patients can be detected by CGH. With recently developed high resolution (HR-CGH) method, we can find aberrations of < 10 Mb.

In our work, CGH was applied to 46 NB specimens and the data were reviewed and correlated with clinical characteristics, including survival analysis. The results from our study confirmed different CGH profiles in the three major clinicogenetic subgroups. Using conventional CGH the highest incidence of genetic imbalances was observed on chromosomes 1p, 2p, 3p, 11q and 17q. In addition, by means of HR-CGH we were able to detect clones with whole or partially chromosome losses or gains occurring with low frequency.

Our results have illustrated the power of CGH/HR-CGH as a sensitive method for the detection of all clinically important genetic alterations in neuroblastoma with good correlation to other relevant methods, e.g. FISH.

P0393. Breakpoint mapping in a Danish patient with otosclerosis and a balanced translocation t(12; 15) (p13.32; q25.1)

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Otosclerosis with a prevalence of 0.2-1% is the single most common cause of hearing impairment. The disease is characterized by isolated endochondral bone sclerosis of the labyrinthine capsule. Conductive hearing loss develops when otosclerotic foci invade the stapedio-vestibular joint (oval window) and interfere with free mobility of the stapes. Sensorineural hearing loss may also be present. Mean age of onset is in the third decade and 90% of affected persons are under 50 years of age at the time of diagnosis. At present, seven otosclerosis loci (OTSC1-7), are known or reserved, but no underlying genes have been identified.

The objective of the present study was to map the breakpoint in a patient with otosclerosis and a balanced translocation. Hearing impairment segregated with the balanced translocation in a brother and the father. At present, we have localised the breakpoint to 15q25.1 and 12p13.32 respectively, by fluorescence in-situ hybridization (FISH) mapping. Interestingly, on chromosome 15 this localization is just proximal to the otosclerosis locus, OTSC1,described by linkage analysis. However the breakpoint is within the DFNB48 deafness locus. We are planning to screen DNA from a panel of otosclerosis patients for mutations in identified candidate genes.

P0394. Partial monosomy 13q syndrome. Report of four unrelated cases.

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Partial monosomy 13q syndrome is an uncommon but well-recognized abnormality of chromosome 13. The wide spectrum and variability of

phenotype manifestations associated with this syndrome depend on how much of the long arm of chromosome 13 is deleted. Here we report four unrelated cases with a de novo partial 13q deletion. Three of them are cytogenetically visible, such as r(13)(p11;q34) found in 3 years old boy, del(13)(q31-32) in 3 months old boy, and del(13)(q22.2-q33) in 2 months old girl. Subtelomeric FISH analysis reveal the fourth aberration, submicroscopic terminal del(13)(q34), in 9 months old girl. Clinical presentation of all cases share large number of common features: pre- and postnatal growth retardation, microcephaly, broad prominent forehead, hypertelorism, small nose, depressed nasal bridge, anteverted nostrils, enlarged mouth with down-turned angels, slight micrognathia, highly arched palate, apparently low set ears, short neck, small hands, thenar hypoplasia, proximal placement of the thumb, clinodactyly of the fifth finger, partial or full simian palmar crease, over-riding toes. All cases present with mental retardation, most profound in ring 13 patient. Brain anomalies are confirmed in three of them and suspected in the fourth. Genitalia are abnormal in the patient with r(13) whereas the subtelomeric terminal deletion case shows anorectal anomaly. This report further contributes to the clinical and genetic delineation of the partial monosomy 13q syndrome in accordance to the size of the deleted region.

P0395. Cytogenetic abnormalities in a case with plasmacytoid dendritic cell leukemia

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CD4(+)CD56(+) malignancies are rare hematologic neoplasms, which were recently shown to correspond to the so-called type 2 dendritic cell (DC2) or plasmacytoid dendritic cells. We present a case of CD4(+)CD56(+) acute leukemia that coexpressed CD 123, HLA - DR , CD 36, CD 38, CD 45RA and was CD 3 negative, showed the typical clinical course of plasmacytoid dendritic cell leukemia, and had unexplained aberrant karyotype. The proband's karyotype revealed absence of one normal chromosome 13 and 21, additional material attached to the short arm of chromosome 20 and two copies of marker chromosome. The additional material on 20p was characterized by FISH, using chromosome paints, as part of chromosome 13. The two copies of marker chromosome were identified as two isochromosomes for 21q using CGH and FISH. FISH analysis also showed the deletion of chromosome region 13q14.3 (D13S25 locus) in 80 % of examined interphase nuclei. We ascertained that the proband's karyotype was 46,XY,-13,der(20)t(13;20)(q12;p12),i(21q),+i(21q).

Our results illustrate the power of FISH and CGH to detect and characterize chromosomal rearrangements that couldn't be solved solely with classical cytogenetic analysis.

P0396. A de novo partial monosomy 12p and a partial trisomy 18q in a chorionic villus biopsy.

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Genomic imbalances can cause mental retardation, congenital malformations and miscarriages. We report about a healthy 36-year-old G3P2 woman who was referred to our hospital for a chorionic villus biopsy at 13 weeks of gestation. Prenatal diagnosis was performed because of an increased risk for trisomy 21 based on serum screening and nuchal translucency measurement. Cytogenetic analysis of G-banded chromosomes showed additional material on the short arm of chromosome 12. Multicolour FISH and FISH using subtelomeric probes revealed a de novo unbalanced translocation, 46,XX,der(12)t(12;18)(p13.3;q21) resulting in a partial monosomy 12p and a partial trisomy 18q. The parental chromosomes were normal. Additional ultrasound screening did not reveal any structural abnormalities. Because of a large imbalance of genetic material, the pregnancy was terminated at 19 weeks of gestation. Post mortem examination of the foetus revealed multiple mild dysmorfological features.

P0397. The familial reciprocal translocation t(2;6) (p21;p25) associated with wide spectrum of phenotypic signs - from normal to severe malformations

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We present a case of familial reciprocal translocation t(2;6)(p21;p25) associated with wide spectrum of phenotypic signs - from normal to severe malformations. A girl who presented with unusual phenotypic signs had a reciprocal translocation 46,XX,t(2;6)(p21;p25), inherited from phenotypically normal father. Our patient is four-year-old girl, the second child of healthy non consanguineous parents from the second complicated pregnancy with symptoms of early toxicosis and miscarriage. The girls' dysmorphic features are presented from the birth: dolichocephaly, coloboma and lid ptosis of the right eye, cleft lip and palate on the right size, lissencephaly. The ulcerative colitis was diagnosed about one year ago. The psychomotor development of our patient is with slight features of delay.

Her clinical findings such as cardiac and abdominal ultrasonography were without pathological changes. Lissencephaly was identified on a cranial ultrasound scan at the early infancy.

The identical translocation has a twin brother of the girl's father too. There are five healthy children and one miscarriage in this twin brother's family.

Cytogenetic analysis of peripheral blood lymphocytes showed the same reciprocal translocation t(2;6) (p21;p25) in girl with severe malformations and her father and father's twin brother are phenotypically normal. Chromosome analysis was performed from GTG banded metaphases. The resolution level was 400-500 bands.

P0398. Parental origin in recurrent trisomic abortions

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Spontaneous abortions (SAs) occur in 10-15% of clinically recognised pregnancies and chromosomal anomalies account for at least 50% of first trimester SAs. Recurrent miscarriages (RM) affect up to 3% of couples trying to have children.The frequency of aneuploidy in RM varies between different studies and has been mostly associated to maternal age.

Here we present six cases with recurrent fetal aneuploidies. Data from chromosomes implicated, parental origin, parental age and total of gestations are shown in the table.

	1st tris (origin)	2nd tris (origin)	3rd tris	Gestations	Mat/Pat age
A	47,XY,+7	47,XX,+22	47,XX,+22	G6/P2/A4	35/37; 39/41; 41/43
I	47,XX,+13	47,XX,+14		G4/P0/A4	36/ ;37/
MM	47,XX,+22 M-I	47,XX,+7 M-I		G3/P1/A2	32/32; 33/33
MC	47,XX,+21 P-II	47,XX,+22 M-I		G2/P0/A2	33/39; 34/40
N	47,XX,+13 M-I	47,XX,+5 M-II		G4/P0/A4	33/34; 33/35
Y	47,XX,+16 M-I	47,XY,+7 M-I		G6/P1/A5	39/40; 40/41

Parental karyotypes were normal. In addition, all couples presented heterotrisomy, which discard gonadal mosaicism. As expected parental origin was maternal in most cases, but a paternal origin was found in one trisomy 21 case. Maternal age was >35 only in half of them and reproductive history showed the presence of further miscarriages (not cytogenetically studied) as well as sterility periods in most of them. Other mechanisms apart from those related to maternal age, like hormonal imbalances, genes involved in chromosomal recombination or environmental exposures might predispose to recurrent trisomic abortions.

P0399. Haematological and Cytogenetic studies on Rheumatoid Arthritis in Tamilnadu state,India.

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Rheumatoid Arthritis is a chronic disease affecting joints and is one of the leading disease types in Tamilnadu state, south India. In the present investigation, we analyzed the hematological and Cytogenetic factors in RA patients. Around 340 patients were selected (males: 160,females: 180) in a population based case control study from Tamilnadu state. In Haematological parameters, we carried out the RBC count, WBC count, Differential Leucocytes count, Erythrocyte sedimentation rate(ESR) and total Hemoglobin(Hb) content. The results were statistically significant in Haematological tests, when compared to the control samples. Chromosomal analysis was carried out using standard Karyotype procedures in 60 male and female patients independently. Among the male subjects only 8 of them (13.33%) displayed chromosomal aberrations and in females 13(21.67%) displayed chromosomal aberrations. In the present study, one male subject with deletion of short arm of chromosome 5[46,XY, del (5p-)] and a female with a deletion of long arm of chromosome 6[46,XX, del (6q-)] was observed .The deletion of the short arm of chromosome 6[46,XY, del (6p-)] was also noted in two males. We also observed other changes like translocations, inversions, satellite formation and mosaic types in different chromosomes of both male and female experimental samples as compared to the controls. The present investigation clearly showed more females affected than males. Also, post menopause females in age group of 45 years and above were affected more when compared to females of lower age groups.

P0400. Meiotic segregation of rare Robertsonian translocations: sperm studies of three t(14q;22q) cases.

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The t(14;22) remains one of the rare Robertsonian translocations observed in human, with an occurrence estimated at 1.2%. Three cases of Robertsonian translocation t(14 ;22) were investigated for meiotic segregation in sperm samples from male carriers using FISH procedure. The 3 carriers included 2 men with an abnormal semen analysis (oligoasthenoteratozoospermia and teratospermia) and one with normal semen parameters. Both locus-specific probes and whole chromosome painting probes, specific for chromosomes 14 and 22, were used in this study. The number of spermatozoa scored for each probe set ranged from 3279 to 10024. In the 3 carriers, similar frequencies were found for normal and balanced spermatozoa resulting from alternate segregation (from 78.53% to 81.76%). The total proportion of unbalanced spermatozoa resulting from adjacent modes of segregation ranged from 17.59% to 20.94%. This finding confirmed the predominance of alternate segregation over other segregation types in all Robertsonian translocations and indicates a higher production of imbalances in the t(14.22) than in most of the Robertsonian translocations previously analysed. This could be related to the variable location of breakpoints in Robertsonian translocations. This breakpoint diversity could also play a role in the differences in reproductive status observed in male carriers of Robertsonian translocations.

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P0401. Fluorescent In Situ Hybridization (FISH) demontrares an interstitial deletion of short arm of chromosome 10 at p11.2p12.32 in a patient with Rubinstein Taybi Syndrome (RTS). New candidate loci for RTS?

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A seven year old Iranian boy with Rubinstein-taybi syndrome (RTS) features was referred to the Genetics Research center for both clinical evaluation and chromosome investigation. The phenotypic abnormalities included CHD, downslanting palpebral fissures, protruding tongue, broad thumbs and toes and moderate mental retardation. Chromosome study by high resolution GTG banding showed a partial deletion of short arm of chromosome 10. The Fluorescent In Situ Hybridization (FISH) was carried out using BAC probes specific for loci within band p14 and PCR probes specific for loci within bands p12.2 and p13 on the short arm of chromosome 10. The resuls indicated an interstial deletion in the short arm of the abnormal chromosome 10. The deleted region is estimated to be between 6.5Mb and 15.5Mb in size. It does not include the GATA3 and BRUNOL3 genes, deletions of which have been associated with phenotypic features similar to Di George syndrome. So far in 10 percent of Rubinstein Taybi cases, deletion of short arm of chromosome 16 at p13.3 has been indicated. To our knowledge, this is the first reported such case which could introduce new candidate loci for RTS.

P0402. Complex mosaic imbalanced karyotype in prenatal diagnosis: identification of small supernumerary marker chromosomes using high resolution multicolor FISH approaches

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The combination of different constitutional chromosomal abnormalities, few small supernumerary marker chromosomes (sSMC) in high level mosaic karyotype is rare cytogenetic finding. We report the results of prenatal karyotyping (amniocentesis in 17 weeks of gestation because of mother's age 38 years) where standard GTG-banding analysis revealed mosaic imbalanced karyotype in fetus 47,XY,der(18),+mar[70]/48,XY,+mar1,+mar2[20]/47,XY,del(18),+mar[5]/46,XY,-18,+mar[5]. Karyotypes of spouses were normal both.

Molecular cytogenetics. We applied M-FISH, high resolution multicolor banding FISH (MCB) and centromere-specific DNA probes for further clarification of prenatal cytogenetic diagnosis. Using material of the next several subcultures of amniocytes the new data of mosaic karyotype were shown by M-FISH: 48,XY,del(18),+mar1,+mar2/48,XY,der(18),+mar1,+mar2/48,XY,+mar1,+mar2/47,XY,der(18),+mar1/47,XY,der(18),+mar2/47,XY,del(18),+mar1/47,XY,del(18),+mar2/47,XY,-18,+mar1,+mar2/46,XY,-18,+mar/46,XY,der(18)/46,XY. Mar1 and mar2 were characterized by M-FISH as micro-ring chromosomes 16 and 18 correspondingly, and sequent FISH study using cep16 D16Z3 SO and cep18 D18Z1 SG (Vysis) confirmed these results. The next step of diagnosis using MCB allowed to specify precisely the deleted chromosome 18 as del(18)(p11.1) and demonstrated the involvement of segments 18p11.2-11.3 in mar2 formation. Small unknown segment of additional material on p arm of der(18) was not originated from chromosome 18.

The mechanisms of de novo formation of the complex mosaic imbalanced karyotype and algorithm of elucidation of origin and extent of chromosomal imbalance in mosaic fetus are discussed.

P0403. An unusual finding in couples with 6 recurrent abortions

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Cytogenetic studies of early spontaneous abortions show a very high rate of abnormality, specially those occurring before 12 weeks of gestation when some 60% show abnormalities.There have been several studies of the chromosomes of couples who have had repeated spontaneous abortions. In more than 10% of such couples one partner is found to have a balanced chromosome anomaly. Chromosome studies among the large number of couples with recurrent abortions show that about 1.5% of the cases were due to the structural chromosomal rearrangements. A 30 years old woman was referred to our Department following to six spontaneous abortions, all during the first trimester. Karyotyping was performed by high resolution banding technique according to standard procedures and showed 46,XY for her husband, and 46,XX,ins(5;4)(q22;q35q22) for our case. Therefore, karyotyping of her family was carried out consisted of her mother,

father and her only brother and the results are as follows:
Mother: 46,XX
Father: 46,XY,ins(5;4)(q22;q35q25)t(4;7)(p15.2;p22)
Brother: 46,XY,ins(5;4)(q22;q35q25)There were 46 chromosomes in all the cells of the father, with an inverted insertion between chromosomes 5 and 4. The long arm segments between bands 4q35 and 4q25 has been inverted and then inserted in to the long arm of chromosome 5 at the band 5q22. There was also translocation between chromosomes 4 and 7 at the band of p15.2 and p22. It is not clear that why this translocation was not in our case and her brother. It is worth to mention that the mother of our case had 14 spontaneous abortions

P0404. Instability of genomes with the patients with streptococcus tonsillitis

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We defined the level of genomes injury in peripheral blood cells at 179 streptococcus tonsillitis patients.
The intensity of aneu-clastogenesis in organism of the patients was valued on the level of micronuclei in erythrocytes and the quantity of aberrations in lymphocytes of peripheral blood.
It was stated, that the level of micronuclei and reconstructions of chromosomes in blood cells of most of the patients significantly ($p < 0,001$) exceeds the same indexes of the healthy people, both in acute period of disease and in the periods reconvalescence (8-10 and 30-32 days from the illnesses onset). The level of genomes injury was correlated with the intensity and frequency of the disease and did not depended on the age and gender of the patients.
It is supposed that destabilization of genome is unspecified stage in pathogenesis of streptococcus tonsillitis.
The revealed phenomenon from the one hand can reflect the high level of generation of endomutagens in the patient's organism and from the other lead to the reduction of activity of the genome protection systems.
To control this suggestion by the method of solution we valued the mutagenic activity of the blood serum, and by the method of culonometric titration we valued the oxidant volume of blood.
The significant decrease of antimutagenic activity and antioxidant volume of blood serum is indicated with the streptococcus tonsillitis patients.

P0405. Array-CGH analysis and clinical description of 2q37.3 de novo subtelomeric deletion

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We report a 13-year-old girl with normal karyotype and a cryptic submicroscopic terminal deletion of chromosome 2q, detected by FISH using Multiprobe-T System (Cytocell Co). FISH analysis of both parents using subtelomeric specific probes for chromosome 2 (Vysis. Inc) revealed that the abnormality is de novo. Further investigation with array-CGH analysis using the 1Mb resolution Spectral Chip 2600 (Spectral Genomics) confirmed the deletion and delineated the breakpoints (2q37.3). Additional FISH studies using bacterial artificial chromosomes (BACs) covering the region 2q37.2-qter, were performed and a deletion of all the probes located in 2q37.3 was found, confirming the results of both FISH and array analyses. Based on FISH and array analyses the breakpoint is located between 236,1 and 238,3 Mb and extends to the telomere. Clinical findings include: developmental delay, severe behavioural disturbances, growth-pubertal retardation, dysmorphic facial features, excessive joint hypermobility, brachymetaphalangism, abnormal dermatoglyphics and a history of neonatal laryngomalacia, hypotonia and umbilical hernia. Hearing evaluation showed congenital conductive mild hearing loss bilaterally, while growth hormone deficiency and compensate hypothyroidism was demonstrated. To date, approximately 60 cases of deletions of chromosome 2q37 (visible or submicroscopic) have been reported with significant clinical variability. The findings in our proband are in keeping with those of the literature, as well as the facial features with the exception of cardiovascular, urogenital, neurological anomalies and eczema which were not observed. The report of the clinical and molecular presentation of similar cases will allow accurate phenotype-genotype correlation and proper genetic counseling of the family.

P0406. An unexpected *de novo* 22q13 subtelomeric deletion - a case report

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We report a cytogenetic investigation of a 3-month old boy, to whom an unexpected *de novo* 22q13 subtelomeric deletion was detected.
He was born prematurely at 36 week of gestation by Caesarean section due to intrauterine growth retardation and oligohydramnion. His birth weight was 1740g (-2 SD), length 45 cm (-1.5 SD), Apgar score 3/6/7. The mother was first time consulted by geneticist at 18 week of pregnancy due to positive double test (AFP 2,62; HCG 2,94 MoM). Ultrasound investigation revealed bilaterally dilated ureters.
At the age of 3 months his clinical picture consists of developmental delay, failure to thrive (-2 SD), bilaterally dilated ureters, bilateral hydrocele, umbilical hernia, hearing impairment from the left ear and persistent foramen ovale. Brain MRI showed hypoplasia of corpus callosum, cavum septi pellucidi and vergae.
The karyotype was found normal by conventional karyotyping (band level 550). FISH analysis for CATCH-22 microdeletion was ordered due to the operated cleft palate present in mother. FISH analysis was done according to the protocol suggested by the supplier of the probes (Cytocell, Aquarius; DiGeorge/VCFS TUPLE1 Region Probe). Surprisingly the analysis demonstrated the absence of the control signal, which locates to the region 22q13.3 and thus the terminal deletion on the long arm of chromosome 22 in all metaphases analyzed. Both parents were normal.
22q13.3 deletion syndrome may not be rare and is still underestimated. The diagnosis of deletion 22q13 syndrome should be considered in children with developmental delay and hypotonia in whom other common etiologies have been excluded.

P0407. Complete gonadal dysgenesis 46,XY in two sisters and their maternal aunt with a female phenotype

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We report two sisters of both with 46,XY karyotype and normal female phenotype (Swyer's syndrome). Swyer's syndrome is a form of pure gonadal dysgenesis characterized by a female phenotype, 46 XY karyotype, hypoplastic gonads, and a normal mullerian system. They appear to be normal females; however, they do not develop secondary sexual characteristics at puberty, do not menstruate, have streak gonads in ovarian localisation, and elevated levels of gonadotropins. These sisters were studied from a clinical, endocrinological and genetic perspective. In 1998, she was at the age of 15 who was presented to us with a main complain of primary amenorrhea. Physical and gynecological examinations, hormonal and chromosomal analyses were performed trying to reveal the etiology. A karyotype revealed 46,XY in 200 GTG-banded metaphases with no detectable mosaicism. She underwent laparoscopic removal of bilateral dysgenetic gonads due to risk of gonadoblastoma development. Her sister at the age of 15, in 2005, was presented to us with primary amenorrhea. Chromosome analyses was performed and 200 GTG-banded metaphases were analyzed. It revealed a 46,XY male karyotype as same as her elder sister. Close follow-up is planned by adolescent gynecology in order to have gonadectomy. There is also observed one case in a sibship of maternal aunt who has same history of primary amenorrhea but married with no consanguinity and have no children yet.
As a proposition we counsel the family for molecular studies of SRY gene and the reason of their localisation far from our university, this study will be made soon.

P0408. A case of complete tetraploidy in Amniotic fluid culture, with normal Karyotype in the repeat

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We report a case of complete tetraploidy in amniotic fluid culture obtained at 14 weeks of pregnancy. Amniocentesis was performed in this pregnancy because of high maternal age and history of offspring with meningomyelocele. Sonography at that time revealed a single fetus with normal fetal activity and heart beat. Amniotic fluid volume was normal. The amniotic fluid obtained was yellow and clear. It was cultured in 2 flasks. Growth was very slow in one culture with no growth in the other. Harvest was possible after 3 weeks which revealed tetraploidy in all studied plates. AFP of amniotic fluid was 24.1KIU/ml (normal range 11.1-48.1 for 15 weeks).
A repeat culture was performed at 18 weeks of gestation and a FISH analysis was performed using X and Y centromeric probes. Repeat culture revealed 46,XY pattern in 89 out of 90 studied plates. Only one plate revealed tetraploidy. 200 interphase cells were studied for the FISH analysis and 98% had one single X and one single Y signal.
Sonography at 18 weeks of pregnancy revealed no abnormality. A healthy male infant was born at term and is doing well.
We conclude that abnormal karyotypes in poor growth cultures could be misleading and have to be confirmed with repeat cultures.

P0409. Rertrospective FISH-analysis of tetraploidy in native extraembryonic tissues of first-trimester spontaneous abortions with 4n or mosaic 2n/4n karyotype after conventional cytogenetics

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It has been found that 3-6% of first-trimester spontaneous abortions with chromosomal abnormalities have tetraploid karyotype. Conventional cytogenetic analysis of abortions from Tomsk population has revealed a high frequency of tetraploidy (22%). In dead embryos tetraploidy usually present in the mosaic state. This situation is considered to be an artifact due to increasing ploidy level during cell culturing, therefore a true impact of tetraploidy on prenatal selection remains unclear. On the other hand, polyploid cells are an obligatory element of human placenta. The aim of the present research was to determine the level of tetraploid cells in native extraembryonic tissues of first-trimester spontaneous abortions with 4n or 2n/4n karyotype by dual-colour interphase FISH with centromere-specific DNA probes for chromosomes 11 and 17. The one-sided upper reference limit for tetraploidy detection in the extraembryonic mesoderm of the placenta (1.0%) was determined in the control group of induced abortions. Thirty spontaneous abortions with 4n or 2n/4n karyotype after conventional cytogenetic analysis were studied. The frequency of tetraploid cells in long-term cultures of mosaic embryos and in non-cultured tissue has varied from 4 to 69% and from 0.3% to 95.2%, respectively. But statistically significance excess of control level was registered for 13 embryos only (43%). Thus the verified frequency of tetraploidy among spontaneous abortions with chromosomal abnormalities is 9.6% in studied population. Our data indicate that cell polyploidization in vitro should be taken into account for analysis of cytogenetic factors of prenatal selection in human. This research was supported by RFBR number 05-04-48129.

P0410. Toriello-Carey like phenotype associated with a complex intrachromosomal rearrangements on 4q.

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Our Units are involved in the diagnosis and the management of syndromic hearing loss.
Here we report on a 3-years-old child who presents conductive hearing loss, evaluated by behavioural audiometry and confirmed by ABR (threshold 60dB nHL), associated with bilateral atresia of external auditory canal, global developmental delay, hypotonia, atrial septal defect ostium secundum and patent ductus arteriosus, cryptoorchidism and peculiar facial dysmorphisms (microcephaly, prominent forehead, telechantus, short and upslanting palpebral fissures, small nose, severe micrognathia, high arched palate, low set, posteriorly shaped and dismorphic ears). The clinical phenotype suggests the Toriello-Carey syndrome (TCS).
The high resolution classical cytogenetic studies and further molecular characterization (using CGH-array and FISH analyses), revealed a complex intrachromosomal rearrangement on the long arm of chromosome 4.
The aetiology of the TCS is still unknown, however an autosomal recessive inheritance seems likely. To date, only three different chromosome abnormalities have been identified in patients with TCS, suggesting genetic heterogeneity. In conclusion, our cytogenetic case report disclosed further genetic heterogeneity in TCS.

P0411. Are parents of trisomic offspring at increased risk for carrying a chromosomal anomaly?

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Karyotypes are often performed for parents of trisomic offspring. This is merely done in order to detect structural anomalies which could increase the risk for aneuploid gametes through interchromosomal effect.
In this retrospective study, we looked at the laboratory files of 316 post- and prenatal cytogenetic analyses with trisomy and registered the parental karyotypes. The aim of the study was to see whether these parents are more likely to carry a chromosomal anomaly compared to the general population.
For the 316 trisomic samples, 309 maternal and 275 paternal karyotypes with G-staining were performed. Of these 584 karyotypes, 577 were normal (98.8%), 1 was numerically abnormal (0.17%) and 6 were structurally abnormal (1,02%). Three structural anomalies were Robertsonian translocations causing the trisomy in the offspring and 3 structural anomalies involved other chromosomes than the trisomic chromosome of the offspring (2 reciprocal translocations and 1 pericentric inversion). If we omit the Robertsonian translocations, this means that a parent of a child with regular trisomy has a risk of 3/581 (0.51%) of being carrier of a balanced structural chromosal anomaly and a risk of 1/581 (0.17%) of having a numerical chromosomal anomaly. When we compare this to figures for the general population (0.15% reciprocal translocations and inversions; 0.15% for sex chromosomal aneuploidy), there seems to be an increase in the risk for structural anomalies, however this study only contains a small number of samples and larger studies should be set up.

P0412. Three months survival of a patient with non mosaic trisomy 22

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We report on a girl who was the fourth child of a healthy, non consanguineous German couple. Two elder sibs are healthy. In the third retarded sister no cytogenetic anomaly was detected. The patient was born at term spontaneously after an uneventful pregnancy out of breech presentation. Amniotic fluid was green, APGAR score 7/7/8. Birth measurements were low normal (weight 2690 g = P3, length 49 cm = P10-25, OFC 32.5 cm = P3-10). Facial anomalies were: marked hypertelorism, left sided cleft lip and cleft palate and low set ears. Further clinical investigations revealed an ASD II and a stenosis of the pulmonary artery, an agenesis of corpus callosum and a dilated renal pelvis.
Cytogenetic analysis of lymphocytes showed trisomy 22 in 100 analysed metaphases (karyotpe 47, XX, +22). Whole chromosome paint 22 confirmed the identity of the additional chromosome 22. Chromosomal analysis of fibroblasts confirmed the diagnosis of trisomy 22 in all 50 analysed cells. Cytogenetic investigations of the parents showed normal karyotypes (46, XX, 46, XY).
The patient needed oxygen supply throughout her life. After intensive care and hospital treatment for one month she lived at home for 2 months and died of intestinal complications (vomiting and constipation). Further investigations and surgical treatment were not wanted.

Survival of patients with non mosaic trisomy 22 is rarely reported. The facial phenotype with marked hypertelorism and cleft lip and palate is recognizable. We will provide a brief review of the previously published cases.

P0413. Non-enzymatic labeling of DNA using the universal Linkage System (ULS ™) in arrayCGH applications

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The Universal Linkage System (ULS™) is a (platinum-based) labeling technology that allows labeling of biomolecules like RNA, DNA and proteins with a large variety of widely used haptens and fluorophores. Because the ULS™ labeling system is a chemical labeling technology, complete control over the labeling process is achieved. The “no need for enzymes” approach makes the labeling procedure very reproducible and robust. For arrayCGH applications the ULS™ system demonstrates one of its key advantages by the direct labeling of genomic DNA samples isolated from both fresh and archival samples. For instance, ULS™ technology makes it easier to obtain high-quality arrayCGH data from old formalin fixed paraffin embedded (FFPE) material.Many arrayCGH applications will require significant amounts of genomic DNA from each sample, the available amount of DNA can be limiting and whole-genome amplification (WGA) is a necessity prior to hybridization. ULS™ offers a flexible and reproducible way to label this WGA amplified DNA. The WGA amplification technology uses unmodified nucleotides which gives higher yields and better quality amplified DNA than with the use of Random Prime methods using modified nucleotides. ULS technology provides a flexible way to label the amplified DNA without a second enzymatic step.This results in a very short labeling procedure with less introduction of bias compared to enzymatic labeling technologies. Here we will report data on the performance of the ULS™ technology in arrayCGH experiments on genomic DNA, FFPE DNA and WGA amplified DNA.

P0414. Molecular and clinical description of a girl with 46,X,t(Y;4)(q11.2;p16)[40]/45,X,der(4)t(Y;4)(q11.2;p16)[10] and a small cryptic 4p subtelomeric deletion.

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A 13-year-old female with clinical features suggestive of Turner syndrome was referred for chromosome analysis. She had short stature, minimal axillary and pubic hair, and no breast development. A CT scan later revealed absence of uterus and ovaries and no masses in the inguinal canal.

Lymphocyte cultures revealed the following karyotype: 46,X,t(Y;4)(q11.2;p16)[40]/45,X,der(4)t(Y;4)(q11.2;p16)[10]. The loss of the small derivative Y chromosome in 20% of the cells was also confirmed in skin fibroblast cultures. FISH analyses using Y centromere, SRY, subtelomere XpYp/XqYq, Y and 4 painting probes, confirmed the cytogenetic findings. High resolution STS analyses using 40 markers spanning the Y chromosome determined no deletion on the Y. However, absence of the 4p subtelomeric region was noted by FISH. Preliminary results with array-CGH analysis using the 1Mb resolution Spectral Chip 2600 (Spectral Genomics) revealed no obvious deletion of the 4p telomeric region, however, the last array clone is 0.35kb distal to the 4p subtelomeric probe used in FISH analysis. Additional array-CGH and molecular studies in the region will reveal the exact breakpoint and the specific genes deleted and will help correlate findings with the clinical presentation.

P0415. Y chromosome mosaicism in Turner syndrome patients.

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Chromosome anomalies accompanying Turner syndrome were found in lymphocyte culture of 230 patients. Chromosomal analysis revealed karyotype 45,X in 117 (50,9%) patients, X monosomy mosaics or structural rearrangements of X chromosome was established in 104 (45,2%) patients. In 9 (3,9%) patients with typical features of Ulrich-Turner syndrome a Y chromosome was found. In 7 mosaics 45,X/46,XY the proportion of XY clone ranged from 46% to 76%. In one of these patients the Y chromosome was dicentric. In most cases of 45,X/46,XY mosaicism, the cause is considered to be the loss of the Y chromosome because of nondisjunction after normal disomic fertilization. In one patient there was apparent nondisjunction of a primary 46,XY conceptus resulting in mosaicism 45,X/46,XY/47,XYY (in 43%, 47% and 10% of cells respectively). In one patient only 47,XYY cells were found (only blood culture investigated). The youngest patient was a newborn baby (mixed gonadal dysgenesis was suspected only for this patient), the oldest was 50 years old. The age of most patients ranged between 15 and 23 years. Mental development of patients was corresponding to their age.

P0416. De novo X/X translocation in a patient with 46,X,ter rea (X;X) karyotype

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X/X translocations are quite rare in man. The effect of this X chromosome abnormality on the phenotype is variable. It depends on the amount of deleted material and whether the chromosomes are joined by their long or the short arms. Our case was an 18 years old girl with 157.5 cm height and 47 kg weight, who was referred to our Department due to primary amenorrhea. Her parents were non-consanguineous and in a good health. She had 4 healthy sibs. In the physical examination, she had manifestations of turner’s syndrome, including: low hairline, short and thickened neck, cubitus valgus, broad and shield-shaped thorax, small breasts, wide-spaced nipples, infantile external genitalia, scant pubic and axillary hair. Sonography report showed lack of ovaries and hypoplasia of uterus. Her IQ was intermediate level. Her thyroid hormones were normal but her FSH and LH levels were high. Her heart, kidneys, eyes, and ears were unremarkable. Metaphase chromosomes were prepared from PHA stimulated lymphocytes. Karyotyping was performed by different banding techniques according to standard procedures and showed the end-to-end translocation or terminal rearrangement leading to duplication of nearly the entire X chromosome. This is a new case report of the Turner’s syndrome which two X chromosomes were joined by their long arms.

P0417. Case report: early recognition of Werner‘s syndrome (WS) on the basis of variegated translocation mosaicism and clonal structural chromosomal rearrangements

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We report a 21-old female patient who was refered to the Tomsk institute of medical genetics with the preliminary cytogenetics diagnosis - 47,XX+mar. She had complaints of short stature and impaired vision. Her examination revealed short stature, bilateral ocular cataracts, corneal leukoma on the left eye, generalized caries, deformation of hard palate, scoliosis, hypoplasia of thyroid gland, hypocalcemia. Standard cytogenetic analysis showed additional small marker chromosomes and multiple translocations involving different chromosomes in 10% metaphases of peripheral blood cells. The following chromosomal rearrangements have been found: 1) 47,XX,t(3;12)(12qter→12q1.4::3p1.4→3qter;12pter→12q1.4::3p1.4→3pter),+mar; 2) 46,XX,t(1;9)(1qter→1q1.1;9pter→9qter::1p1.1→1pter), t(9;15)(9pter→9qter::15q2.1→15qter;15pter→15q2.1); 3) 46,XX,der(19); 4) 48,XX,del(10)(q2.4→qter),+1mar,+2mar; 5) 46,XX,der(4); 6) 48,XX,del(2)(pter→p1.2),+1mar,+2mar; 6) 46,XX,der(13); 7) 46,XX,t(10;14)(10pter→10qter::14q2.1→14qter;14pter→14q2.1); 8) 46,XX,add(17)(q25.3) ; 9) 46,XX,der(18); 10) 46,XX,der(22). The cell clone with t(3;12) was confirmed by CISS with WCP3. The major cell clone was 46,XX. In view of clinical and cytogenetics findings we considered that our patient had WS. Further analysis including molecular testing of *WRN* and *LMNA* genes is warranted and are being performed. To date there are only several cases of comprehensive description of chromosome abnormalities in cultured lymphocytes observed in WS.

P0418. Derivative X inactivation in a girl with unbalanced X;3 translocation and very mild phenotypic abnormalities

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X chromosome inactivation is a mechanism to compensate gene dosage by silencing most of the genes on one of the X chromosomes in mammalian females. It occurs randomly during the early phases of cell differentiation. The inactive X shows a late replicating behavior.

We have studied the spreading of X inactivation in a case of unbalanced X;3 translocation presenting very mild phenotypic alterations. The proband was a girl with a partial trisomy of the q arm of chromosome 3, including the 3q25-qter region, translocated to the p arm of one chromosome X. Xp subtelomeric region of the der(X) chromosome was deleted. The translocation was of maternal origin. The girl presented with mild abnormalities such as short stature, relative macrocrania, limb shortening, pelvis malformation and cholestasis, but no mental retardation.

Methylation analysis at the androgen receptor locus showed completely skewed X inactivation in the proband lymphocytes. A fluorescent BrdU assay combined with in situ hybridization using whole chromosome 3 painting demonstrated that the der(X) chromosome was late replicating in 100% of the analyzed metaphases and the late replicating region extended from the X chromatin across quite all the autosomal fragment excluding only the 3q29 band.

The pattern of gene silencing on the translocated chromosome correlates well with the attenuation of the clinical phenotype associated with 3q25-qter trisomy. Short stature and limb shortening in this case might be ascribed to SHOX haploinsufficiency more than to the trisomy.

P0419. Unique an X;Y insertion in infant 45,X male

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The X-Y translocations are rare cytogenetic findings. We report a unique case with X;Y insertion in 45,X male. He is an infant with prominent congenital progressive hydrocephaly associated with lobar holoprosencephaly, ichthyosis and severe mental retardation. Conventional chromosome investigation performed on cultured lymphocytes with GTG- and QFH-staining revealed mos 45,der(X)ins(X;Y)[41]/45,X[9] karyotype. FISH analyses were performed on lymphocyte metaphase spreads using standard protocols with following DNA probes: X-chromosome (DXZ1), Y-chromosome (DYZ3) centromeres, heterochromatic region Y (Yqh), whole painting probes of Y-chromosome (WCPY), X-chromosome (WCPX) and short arm of X (PCPX). In 82% metaphases in situ hybridization demonstrated presence of X-derivate inclusive Y-chromosome material in locus Xp22 and not revealed any numerical gonosome mosaicism. Molecular analysis was performed using multiplex PCR amplifications of SRY, AMG/AMGL, ZFY/ZFX loci and fifteen Y-specific STSs. Presence of SRY, AMG, ZFY/ZFX, sY2062 and sY1248 have been demonstrated. Yp breakpoint has been localized between sY1248 and sY211 (intervals 1B-2A). X-chromosome hemizygoty have been confirmed by analysis of AR gene CAG-repeats in exon 1, and six additional X-chromosome markers: DXS1062, DXS1192, STR44, STR45, STR49 and STR50. Apparently, the insertion of Y-chromosome material has been occurred in Xp22.3 nearby STS gene.

P0420. Partial Xp trisomy with functional Xp disomy due to an unbalanced translocation between chromosome Xp and 17p

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Functional Xp disomy due to an unbalanced translocation between the short arm of the X chromosome and an autosome is uncommon. Seven cases have been reported. Most of them result from additional segment Xp joined to the proximal short arm of an acrocentric chromosome (13, 15 or 22). Only one observation is related to unbalanced segregation of a maternal (X;16) translocation. We describe a 2-year-old girl with severe hypotrophy (weight -4 SD, lenght -1.5 SD and OFC -2.5 SD), developmental delay with hypotonia, poor head control, seizures and facial dysmorphism including prominent metopic suture, high forehead, strabismus. Her karyotype was 46,XX, der(17)t(X;17)(p11.4;p13.3), resulting from the meiotic malsegregetion of a balanced maternal reciprocal translocation. The partial trisomy of Xp11.4→pter was confirmed using BAC specific probes of the short arm of chromosome X. The additional Xp segment translocated to 17p is not subject to inactivation due to its physical separation from the X-inactivation center at Xq13.2. Thus, the proband presents an Xp trisomy with a functional Xp disomy. Further studies with subtelomeric 17p probe (Totelmix8, Vysis), with DNA Miller-Dieker probe (Vysis) and with BAC probes specific for the 17p13.3 region showed a deletion of the 17p telomeric region without deletion of the Miller-Dieker region. The size of the deletion on the der(17) was evaluated at 1 Mb. The comparison of clinical features observed in the present case and in the cases reported in the literature reveals common phenotypic features but the involvement of the 17p deletion in the phenotype can also be discussed.

P0421. An XX male with the SRY region inserted in the long arm of chromosome 16

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We here present a case of an infertile 46,XX male, who has normal masculinization of the external genitalia. With FISH, using a SRY (Sex-determining Region Y chromosome) gene containing clone, we observed the presence of the SRY gene at the telomere of the long arm of chromosome 16. Further investigation using FISH with BAC and PAC clones revealed insertion of a fragment of 600 Kb of the Y chromosome containing the SRY gene and part of the pseudo autosomal region, at 16qter. The majority of classical XX males have the SRY gene, despite the fact they lack the Y chromosome. In these XX males the SRY gene, which is located close to the pseudo autosomal boundary on Yp11.3, is transferred from Yp to Xp. This is the result of an abnormal exchange during paternal meiosis I between the pseudo autosomal region of the Y chromosome and the PAR1 region of the X chromosome. In our patient most probably an insertional translocation between Yp and 16q during parental gametogenesis gave rise to the aberrant chromosome 16. As far as we know this is the first report of a 46,XX male with the SRY gene transferred to an autosome.

P0422. XYY in mentally retarded boy with tall stature, prognathism, hypoplastic toe nails and malformations of the hands

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Here we report a 25-year-old man referred to our laboratory for evaluation of possible Fragile X syndrome on the basis of mild mental retardation. He was tall (194cm). His face was mildly dysmorphic. Prognatism was detected. He often showed excess negative mood and aggressiveness. He could finish his primary school. He worked as a mechanic. He suffered from Spasm and muscle cramp. He had hypoplastic toe nails and short hands. Although he had a hydrocoele repaired, his genitalia were otherwise normal. The patients found to be negative for Fragile X. However, He was found to be 47 XYY from chromosomal examinations.Our case is the second reported case of a XYY boy with malformations of the hands. We could find just one previous article concerning XYY males with hands malformation. This combination of XYY male and nails and hands deformities may be fortuitous. However, we think it is important to report the patient.

In summary, it is apparent that the mental and physical characteristics of the average XYY boy are yet to be determined. This is very important

to help the parents who are seeking prenatal diagnosis and whom found to have a fetus with XYY karyotype to make the right decision.

P0423. Cytogenetic and molecular characterization of Y chromosome abnormalities coupled with infertility

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Infertility affects approximately 15% of all couples. The male partner is involved in about half of the cases. Chromosomal abnormalities are frequently observed in infertile men. Among infertile men, the prevalence of microdeletions of the Y chromosome is approximately 7%. Most of the Y chromosome microdeletions occur on the long (q) arm and involves the azoospermic factor (AzF) regions: AzFa, AzFb and AzFc.

The aim of this study was to estimate the incidence of Y chromosomal alterations in patients enrolled for assisted reproduction. Preoperative evaluation includes andrological investigation of the semen, testicular ultrasound, and analyses of hormone levels. To establish the Y chromosome abnormalities, cytogenetic analyses were also performed, in one female, and in 38 infertile men either with azoospermia or with severe oligozoospermia. In order to establish an exact diagnosis, the traditional and modern cytogenetic methods were combined with molecular genetic techniques.

We found patients with 48,XXYY, 47,XYY-syndrome, a female with 46,XY partial gonadal dysgenesis with bilateral dysgenetic testis. Two men with Y chromosome microdeletions were found. One of them was mosaic 45,X/46,XderY, where the Y chromosome was missing in more than 90% of the cells. In the rest of the cells deletions were detected in the AzFb and AzFc regions of the Y chromosome. In the other case, a non-mosaic Y chromosome was observed with deleted AzFc region.

The incidence of chromosomal anomalies among infertile patients strongly suggests the necessity of genetic investigation and counseling prior to the ICSI treatment.

P0424. Use of Chromosome microdissection for Preparation of bandicoot Y chromosome-specific FISH paint: a tool for the study of sex chromosome elimination in mammals

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Sex chromosome elimination is rare among mammals. X chromosome elimination occurs in some species of eutherians, but Y chromosome elimination from somatic tissues has been seen only in marsupials. Among marsupials, the bandicoot *Isodoon macrourus*, is of particular interest, since this animal is very immature at birth, and during pouch life sex-chromosome loss occurs at different stages of development in different tissues. In the present investigation, the technique of chromosome microdissection and linker-adaptor PCR was developed to produce Y specific paint from bandicoot *Isoodon macrourus*. By employing Y-specific paint it should now be possible to examine interphase nuclei and precisely determine the proportion of cells that eliminate the Y chromosome from different tissues of this animal. The Y specific paint prepared here would be particularly useful for studying Y chromosome elimination in haematopoietic cells from different tissues and blood during early bandicoot development.

P0425. AZFc deletion in 45,X/46,XY/47,XYY/48,XYYY male

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Yq deletions are common cause of X/XY mosaicism owing to Y chromosome instability. We report on the Y-chromosome long arm deletion in a 15-year-old mosaic 45,X/46,XY/47,XYY/48,XYYY male. He had male external genitalia with sufficient masculinization, short stature (151 cm) and some other Turner stigmata: hypersthenic, mandibular hypoplasia, broad shield-shaped chest with wide-spaced nipples, moderate short legs and arms. In anamnesis patient had penal hypospadia that was corrected by surgery. The cytogenetics examination was carried out on leukocyte metaphases from peripheral blood using GTG- and C-staining according to a standard method. Dual-color FISH was performed with CEP X Spectrum Green and CEP Y Spectrum Orange labeled DNA probes. DNA was extracted from peripheral leukocytes. DNA amplifications of SRY, ZFY/ZFX and 12 Yq-specific STSs: sY86, sY84, sY615 (AZFa); sY127, sY134 (AZFb); sY142 (proximal AZFc border); sY254, sY255 sY1197, sY1192, sY1206, sY1291 and sY1125 (AZFc) were performed using two multiplex PCR. Patient's karyotype revealed by conventional chromosome analysis is mos45,X[20]/46,XY[3]/47,XYY[1]. Fluorescence in situ hybridization had show that the percentage of interphase cells with only X-signal was 72% [645], XY - 18%[170], XYY - 9%[80], XYYY - 1%[5]. All analyzed AZFc region specific markers were absent. Molecular analysis not revealed any Y-chromosome microdeletion in patient's father. Evidently that in proband AZFc region was deleted in all Y-bearing cell lines. To our knowledge, this case is first report with AZF deletion in mosaic Y chromosome polysomy.

P0426. Y/autosome translocation involving satellite region of unknown acrocentric chromosome in a case with oligospermy

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Y/autosome (Y/A) translocations have been described in association with male infertility. Here we report a case of 38 years old man with oligospermy and a rare Y/autosome translocation, involving satellite region of unknown acrocentric chromosome. Routine cytogenetics found an abnormal chromosome Yq consisting proximal to distal of i) an euchromatin region, CBG negative; ii) a satellite region of acrocentric chromosome, CBG positive; and iii) satellite fibbers stained positive by Ag-NOR. The origin of the acrocentric chromosome involved in this Y/A translocation was not found. Molecular analysis of the genes ZFY (Zing Finger Protein, Y-linked) and SRY (Sex Determining Region Y) as well as the polymorphic markers sY254, sY84, sY127, sY86, sY134, sY255 showed no deletion, pointing out that chromosome loci Yp 11.3, Yq 11.1, Yq11.21, Yq11.222, Yq 11.223 were present. However, a very small deletion of Yq euchromatin, distal to Yq11.223 was suspected because of the clinical data of oligospermy. This case report further contributes to the clinical and genetic delineation of Y/A translocations and the rare aberrations involving satellite regions of acrocentric chromosomes. It could be an useful source for future studies aimed at the identification of novel gene(s) for spermatogenesis.

P0427. Turner phenotype and a male pseudohermaphroditism in a girl with 45,X/45,X,t(Y;13)(q11;p13) karyotype.

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The presence of chromosome Y is found in about 6% of cases with mosaic form of Turner syndrome. Here we report a 16 years old girl with a typical Turner phenotype, features of a male pseudohermaphroditism and a chromosomal mosaicism including Y chromosome in one of the clones. Routine cytogenetics performed on lymphocytes found a karyotype 45,X/45,X,t(Y;13)(q11;p13) with a clone proportion of 84% / 16%. Fluorescent in situ hybridization analyses confirmed the presence of chromosome Y. Surgery carried out for gonadoblastome revealed a presence of both male and female internal genitalia. This case report further contributes to the clinical and genetic delineation of the rare chromosomal mosaicism of monosomy X with a second cell line containing abnormal chromosome Y.

P0428. Long-term survival in infant with triploidy - a case report

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Triploidy is one of the most common chromosomal aberrations characterized as a complete extra set of chromosomes that may be maternal or paternal in origin. Triploidy usually results from one out of

two main mechanisms: aberrant segregation of chromosomes during meiosis leading to a diploid egg or the fertilization of one egg by two sperms. Triploidy occurs in 1−3% of conceptuses, but about 99,99% are lost as first−trimester miscarriages or second−trimester fetal death in utero. It is estimated that the incidence of triploidy is about 1:10.000 in live born, but in most patients diploid/triploid mixoploidy is diagnosed. Most patients with full triploidy have died in the early neonatal period. The main clinical symptoms of triploidy are as follows: severe intrauterine disproportional growth retardation, body asymmetry, dysmorphic features and additional congenital defects. We report a very rare case of a triploid newborn with multiple congenital defects who survived to 56th day of life. The triploidy was diagnosed by cytogenetic examination in three type of cells: trophoblasts, lymphocytes and fibroblasts (69,XXY). The patient presents characteristic pattern of triploidy such as: intrauterine hypotrophy, body asymmetry and disproportion between growth of the skeleton and cephalic region, thorax, cardiac and kidney defect, plenty of dysmorphic face features and hands and feet abnormalities. The mechanic ventilation was applied, but patient didn't require breathing stimulation from the 42nd day of life. His clinical status was stable until the 52nd day of life when the severe deterioration occurred with renal and liver insufficiency. The patient died on the 56th day of life.

Po03. Prenatal diagnosis

P0429. Reporting on the outcome of more than 2000 β-thalassemia prenatal diagnosis in Iran.

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Objective: Beta-thalassemia is the most common hereditary blood disorder in Iran. More than 1500 prenatal diagnosis had been completed by our lab since mid 2000.**Material and methods:** Besides routine ARMS-PCR methods, which were used for screening the most common Iranian β-globin gene mutations, more rigorous molecular analyses were performed for the 75 unresolved cases (75/1524 or 5%) using DNA sequencing. **Results:** The total of 21 rare β-globin gene mutations was found, including: 5'UTR -101 (C>T), 5'UTR -88 (C>A), 5'UTR -87 (C>G), 5'UTR -30 T>A, 5'UTR -28 (A>C), 5'UTR +22 (G>A), Cd 15 TGG>TAG, Cd 15 TGG>TGA, Cd 16 (-C), Cd 25/26 (+T), IVSI nt128 (T>G), IVSI nt130 (G>C), Cd 37 TGG>TGA, Cd 37/39 (-GACCCA), Cd 44 (-C), Codon 80/81 (-C), Cd 82/83 (-G), IVSII nt848 (C>A), IVSII nt850 (G>C), IVS-II nt850 (G>T) and Cd 126 GTG>GGG. There were 5 new rare mutations [i.e. -30 T>A, Cd 37/39 (-GACCCAG), Cd 37 (G>A), Cd 44 (-C), IVS-II nt850 (G>T) and IVS-II nt850 (G>C)] which had not been reported in Iran, previously. Also, we found codon 2 CAC>CAT (His>His) and IVS-II nt666 T>C -the two beta-globin gene SNP sites- which the frequencies of rare alleles in these sites were approximately 5% (Cd 2 "T" allele) and 12% (IVS-II nt666 "C" allele), respectively. **Discussion:** Knowing mutation types, their frequencies and hematological data, which were found by us here, could be useful for developing β-thalassemia molecular screening plan in Iran and other neighbor's countries.

P0430. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: a two years review prospective experience in a single center in the Czech Republic

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Last years has intensified the effort to develop early non-invasive methods to screen for trisomy 21 and other main chromosomal abnormalities in prenatal diagnosis. In so-called OSCAR clinic, maternal age, fetal nuchal translucency, as well as maternal levels of ß-hCG and PAPP-A are used as screening markers. Absence of the nasal bone and tricuspid regurgitation have been introduced as a new ultrasound markers in the first trimester that will increase the detection rate of trisomy 21 up to 97% for a false positive of 5%. We report our experience of combining these biochemical and ultrasonographic markers in 2110 pregnant women in our center. The specific Down syndrome risk was calculated by using the Fetal Medicine Foundation software. Karyotyping was offered to women with risks ≥ 1 in 300. On the bases of maternal age of the screened population, 6.5 Down's syndromes and 6.5 other chromosomal abnormalities were to be detected. Ten out of the expected 7 Down's syndromes and 8 out of the expected 7 other chromosomal abnormalities were found resulting in a detection rate of 100% with a false positive 5% (109/2092). The population screened showed 16% aged 35 and more. After the introduction of the first trimester screening to our clinic, the number of invasive genetic testing decreased from 16% to 5%. In our experience first trimester screening for trisomy 21 and other aneuploidies has a high sensitivity with a low false positive rate and can be delivered in an efficient manner in a one-stop multidisciplinary clinic.

P0431. Maternal serum screening for Down and Edwards syndromes and for neural tube defects. Importance of a-fetoprotein

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A- fetoprotein (AFP) is one of serologic markers for 21 and 18 trisomies and neural tube defects screening.

The purpose of the study was to analyse the reliability of this marker between pregnant woman of different age.

Material and method: the analysis was retrospective. The data of 82 patients were reviewed during 2004 year. The age of woman was between 16 and 43 years. The α-fetoprotein quantity in IU/ml and μ/μ0 was evaluated from 14 to 20 week of pregnancy and a comparison with newborn diagnosis was made. According to data from Merkatz and others in 1984 AFP μ/μ0 is about 0.70 in case of Downe and 0,65 in case of Edwards syndrome.

Results: in 65 cases results of AFP were normal and newborns were healthy. In 13 cases AFP was lower than normal (μ/μ0 < 0,75). But newborns were healthy too. In 4 cases AFP was higher then normal (μ/μ0 > 2,0). 3 newborns were healthy and in 1 case a left-sided cheilognatoschisis and cleft palate was diagnosed.

Conclusion: AFP correctly predicted normal outcome in 79% of cases. Normal AFP values are adapted to pregnant woman more than 35 years old. A younger age was observed In 13 out of 17 women with AFP results higher or lower than normal. Normal AFP values for this group of women might be different. Whether cheilognatopalatoshisis associated with higher AFP needs further study.

P0432. Comparison of multiplex PCR and real time PCR for the noninvasive determination of fetal gender from maternal plasma

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Reliable gender determination in early pregnancy generally requires sampling of fetal cells via invasive procedures that are associated with low, but definite risks for fetus and mother. In families with X-linked recessive genetic disorders it is important to early determine fetal gender, thus the development of safer methods is needed.

The purpose of this study was to compare reliability of miltiplex PCR and real-time PCR methods for the determination of the presence of fetal Y-chromosome DNA in maternal plasma. Cell-free DNA was isolated from 43 maternal plasma samples and analyzed using AmpFl STR® Identifiler™ kit (15 STR loci and gender-specific amelogenin) and Quantifiler Y Human Male real-time PCR kit. Gender of fetuses was confirmed by cytogenetic analysis. Amplification of paternal STR alleles was expected to serve as a positive control for the presence of fetal DNA in maternal plasma in case of female fetuses, but unfortunately in vast majority of samples amelogenin was the only fetal locus that was successfully amplified. Out of 26 male fetuses, the presence of Y-chromosome DNA in maternal plasma was successfully determined in 25 of them using both methods (96,2%). There were no falsely positive detected Y-chromosomes in female fetuses, but the results were partly inconclusive because we were not able to eliminate the possibility that the lack of male amelogenin was simply due to inadequate amount of fetal DNA. Further work on this method should consider use of different markers that will be more confirmative of the presence of fetal DNA in maternal plasma.

P0433. Development and application of PCR-STR method for rapid prenatal diagnosis of chromosomes 21 and 18 aneuploidies

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The aims of the present study were: (I) to investigate the heterozygosity and informativeness of six Short Tandem Repeat (STR) loci among Croatians: 3 loci are located on chromosome 21 (D21S1435, D21S1411, D21S1414) and 3 loci on chromosome 18 (D18S51, D18S858, D18S535); (II) to evaluate diagnostic power of these 6 STRs and efficacy of using PCR-STR method for rapid prenatal detection of trisomies 18 and 21; (III) to compare results obtained with PCR-STR method with those obtained by classical cytogenetic analysis.

DNA was isolated from blood (N=205) or amniotic fluid (N=699) by Nucleospin isolation kit. After PCR amplification by Cy5 labeled primers samples were run on a 6% polyacrylamide gel in an automated DNA sequencer (ALFexpress, Pharmacia-Biotech). Fragment sizes were analyzed with AlleleLocator software.

The results indicate that all 6 tested loci are informative and useful in prenatal detection of aneuploidies in our population. Observed heterozygosity was in the range 0.65 - 0.85. In total of 699 samples of uncultured amniotic fluids chromosomal abnormalities were observed in 22 (3.15%) samples (16 samples with trisomy 21, five samples with trisomy 18 and one sample with triploidy). All results were consistent with conventional cytogenetic analysis.

Results from this study clearly demonstrate the high diagnostic value of PCR-STR method with this particular set of STRs for the prenatal detection of numerical disorders on chromosomes 21 and 18.

P0434. Clinical evaluation of the Devyser QF-PCR kit for prenatal diagnostics of chromosomal aneuploidies

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In an effort to improve pregnancy management and soothing maternal anxiety, Quantitative Flourescent PCR (QF-PCR) amplification of Short Tandem Repeat (STR) markers has successfully been introduced as a rapid technique for aneuploidy testing of the most common disorders in prenatal samples.

The Devyser QF-PCR kit is a new CE-labelled product for prenatal diagnosis facilitating aneuploidy testing of the most common disorders in prenatal samples.

The kit contains ready-to-use reagents necessary for the complete analysis. Seven markers each for chromosomes 13, 18, 21 and 10 markers for chromosomes X and Y are included and tested in the standard kit configuration. The high number of markers used in the kit minimises the need for costly and time consuming re-runs of samples

This clinical evaluation of the Devyser QF-PCR kit for prenatal diagnostics was performed in order to assess the clinical usefulness. Archival samples and a prospective series of amniotic fluid samples were collected and analyzed according to instruction for use. The data obtained from the Devyser kit was compared to corresponding data from karyotyping for all samples in order to determine the sensitivity, robustness and usefulness of the kit. In addition, a cohort of blood donor samples were also tested in order to further establish the informativity of the different markers used in the kit.

P0435. Dysfertile couples with recurrent aneuploidies

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There have been published articles in recent scientific literature concerning cases of recurrent aneuploid or polyploid embryos and fetuses in the same couples. Most of studied supernumerary chromosomes were maternal meiotic origin; only seldom they were ascertained as paternal or postzygotic. The association between increasing maternal age and the frequency of aneuploid embryos or fetuses has been known for years - the term "ovarian aging" is used for this process and it is related to decreased oocyte pool in ovaries of older mothers. There are differences among women of the same chronological age: it means risk of trisomic conception depends on biological age of the woman. Aging and premature aging hypothesis has supposed the range of internal and external causes but no of them has been certified.

We present a family in which one healthy son was born and then only unsuccessful pregnancies followed: one termination after prenatal diagnosis and three spontaneous abortions. Three times cytogenetic examinations were provided, every time with abnormal fetal / embryonal karyotype, at maternal age 34,35, 36 years respectively; the father was 12 years older. The origin of supernumerary chromosomes was studied to help the family because there is a possibility to undergo IVF using donor gametes.

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P0436. Combined 1st trimester screening with aneuploidy risk evaluation according to the degree of analyte deviation

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The possibility to increase the efficacy of combined 1st trimester screening by aneuploidy risk evaluation according to the degree of analyte deviation was tested. PAPP-A and free beta-hCG were examined by Kryptor system with LifeCycle Elipse software with our NT/analytes MoMs. Results were arranged into 4 categories according to MoMs: I. (0.6-1.9) in 46.2%, II. (0.5-0.6 and / or 1.9-2.0) in 11.7%, III. (one analyte/NT 2.0) in 31.6% IV. (both analytes abnormal with/ without abnormal NT) in 10.7%. Altogether 1415 pregnancies were karyotyped. No aneuploidy (0/688) was found in I. In II. were only heterochromosomal aneuploidies(2/163 - 1.23%). In III. 3 autosomal and 2 heterochromosal aneuploidies (5/468 - 1.1%) and in IV. 9 aneuploidies (9/96 - 9.4%) were found.

Severe aneuploidy risk corresponded to the analyte deviation (for category IV. p= 0.00001). The software revealed 6/10 of aneuploidies (3/5 of +21, 2/3 +13, 1/1 triploidy without trisomy 10 mosaicism detection). Analyte deviations disclosed 8/10 of autosomal aneuploidies, while NT 5/10 aneuploidies. No autosomal aneuploidy was missed by this strategy. Only analyte deviations revealed all (7/7) heterochromosomal aneuploidies and FraX A syndrome with 4.5 MoM of beta-hCG. Combined first trimester screening with evaluation of analytes deviations revealed all pregnancies with severe autosomal and all heterochromosomal aneuploidies. Genetic counselling is recommended for categories II-IV with second trimester screening, ultrasound and obstetrical controls to improve the screening efficacy and prenatal care. AmnioPCR with 100% diagnostic reliability is performed in categories III-IV to minimise the parental anxiosity.

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P0437. Prenatal Diagnosis of autosomal recessive polycystic kidney disease (ARPKD) without DNA from an index patient

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Autosomal recessive polycystic kidney disease (ARPKD) is associated with poor outcome in affected children. Therefore, prenatal diagnosis is of great importance. Mutational analysis is not a routine analysis in prenatal diagnosis: because of the large size of the PKHD1 gene, linkage analysis is the preferred method. Two families were reported with prenatal diagnosis of ARPKD based on PKHD1 mutation analysis without analysis of material of the affected child. Absence of material from the index case or unclear diagnosis prompted mutation analysis of the parents. Detection of a mutation in both parents offered the possibility for prenatal diagnosis in subsequent pregnancies.

We report on a consanguineous Iraqi couple (niece and uncle) referred for genetic counselling and prenatal diagnosis of ARPKD

at the 12th week of gestation. The first child died 20 minutes after spontaneous premature delivery at 36th week of gestation. Autopsy showed characteristical signs of ARPKD. No biological material was preserved.
Molecular analysis by sequencing of the PKHD1 gene in both parents was initiated with the purpose to perform prenatal diagnosis in following pregnancies if the result indicated a heterozygous mutation in both parents. The couple was advised that, unless mutations would be found in both parents, ultrasound may be the only diagnostic tool in the current pregnancy.
Following the detection of the same novel heterozygous nonsense mutation p.S976X (c.2927C>G) in exon 27 in PKHD1 gene in both parents at the 16th week of gestation, prenatal diagnosis was performed in the current pregnancy without index-patient (with the result of heterozygosity).

P0438. Autosomal trisomies - a very important cause of early spontaneous abortions

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Spontaneous abortion is defined as the termination of pregnancy before 20th weeks of gestation or below a fetal weight of 500 grams. A total of 321 cases of first trimester spontaneous abortions between 4 and 13 weeks of gestation were analyzed cytogenetically by the direct - preparation method using chorionic villi. A total of 172 (54%) of the specimens were chromosomal abnormal, and 91% were numerical chromosomal abnormalities. The most common abnormalities are trisomies, arising de novo as a result of meiotic non-disjunction during gametogenesis in parents with a normal karyotype. Gonadal mosaicism for trisomies can contribute to recurrent trisomy.
Autosomal trisomies comprise 34% of karyotyped spontaneous abortions, and the vast majorities were single trisomies. Trisomies for all chromosomes, with the exception of chromosomes 1, 5, 11, 17 and 19 were observed. Double trisomies [48,XX,+2,+16; 48,XX,+16,+18; 48,XX,+21,+22] and mosaic trisomy [46,XY/47,XY,+7; 46,XX/48,XX,+7,+16; 47,XX,+20/48,XX,+7,+20; 47,XY,+14/48,XXY,+14] were rare. The most frequent was trisomy 16 (34%), followed by trisomy 22 (11%) and 21 (9%). When abortions were stratified by maternal age, 23% of cases were trisomic in women younger than 35 years of age, and 57% were trisomic in women 35 years or older. This study confirms that the incidence of trisomy increases with maternal age. The mean gestational age was 8-9 weeks.
A trisomyc karyotype in chorionic villi from a spontaneous abortion explains the reason for the loss and does not place the couple at higher risk for repeated chromosomal trisomy and pregnancy loss.

P0439. Prenatal diagnosis of the twins at risk of Beta-thalassemia major in Iran.

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Objective: Beta-thalassemea is the most common hereditary blood disorder in Iran. Prenatal diagnostic (PD) of beta-thalassemia is one of the most effective means for preventing the birth of affected children. More than 1256 PNDs have been performed in this laboratory, since mid 2000. The Beta-globin gene analysis of dizygotic twines (fraternal twines) is one of the complexes of PD since there is problem in CV sampling and PD should be performed for two fetuses. **Materials and Methods:** Prenatal diagnosis has been performed for 9 cases of twins so far. We used combined ARMS and RFLP methods to detect mutations. **Results:** Of these, in 8 (8/9 or 89%) cases both ARMS and RFLP methods could help us to detect the pattern of mutation inheritance. In the remaining case the mutation was detected by ARMS method, but RFLP analysis was semi-informative (only one of the parental mutant alleles could be traced by PFLP analysis). **Discussion:** Three out of 10 cases were most probably identical twin since they had similar mutation and RFLP pattern. In the rest the twins were not identical since they had either different PD result (5cases) or had different RFLP pattern (2cases) or they had different RFLP pattern (2 cases) or they had different sexes or VNTR result (1 case each).

P0440. Cell-surface-bound fraction of cell-free fetal DNA in maternal blood as a new marker for non-invasive prenatal gender detection

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It was detected that cell-free fetal DNA circulates in the plasma and serum of pregnant women. This discovery served as incentive to development of new approaches for noninvasive prenatal diagnosis. One of the first such studies was directed to Y-chromosome sequences revealing in maternal blood to diagnose X-linked disorders. It has been shown that sensitivity of Y-chromosome loci detection in maternal plasma by conventional PCR varies from 50 to 95% in different studies in the first trimester of pregnancy. It was recovered recently that there are cell-surface-bound nucleic acids that form the main part of cell-free DNA and RNA besides free circulating fraction in blood. In the present study we realized the PCR analysis of fetal *SRY* gene in maternal plasma and in cell-surface-bound fraction of cell-free DNA as well as estimated possibilities of using this new marker as additional source of fetal DNA for early noninvasive diagnosis of fetal gender. In our study the sensitivity of fetal *SRY* locus detection in maternal blood amounted 100% in 10 cases of male fetus carrying and 2 false-positive signals have been registered in the blood of 5 pregnant women who carried female fetuses. It is significant to note that in 2 women carried male fetuses *SRY* locus was revealed only in the cell-surface-bound fraction of cell-free DNA while no signal was found in maternal plasma. Thus the use of cell-surface-bound fetal DNA allowed raising the informativeness of fetal gender prenatal detection approach up to 20%.

P0441. Prenatal diagnosis and normal outcome of a 46,XX/46,XY chimera

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Here, we report on a new case of chimerism (46,XX [90%]/ 46,XY[10%]) diagnosed at 17 weeks' gestation on amniocentesis performed because of advanced maternal age. The diagnosis was confirmed by karyotype analysis of fetal lymphocytes. Ultrasound examination revealed normal female genitalia. At term, a healthy baby girl was delivered, without any abnormality of the external genital organs.
By definition, chimerism is produced by fusion of two different zygotes in a single embryo, while mosaicism results from mitotic errors in a single zygote. Several mechanisms of production of chimera have been proposed in literature. Stricto sensu, chimerism occurs from the postzygotic fusion of two distinct embryos leading to a tetragametic chimera. In addition, there are two other entities which are by extension, also referred as chimera: parthogenetic chimera and chimera by fertilization of the second polar body.
We used polymorphic markers spanning chromosome X and several autosomes in order to identify the genetic mechanism involved in our case. Mosaicism was excluded because of the presence of three alleles at 12 loci. The origin of this third allele was maternal for one marker (located on band 8p11.2) and paternal for the other markers. Thus, two different paternal haploid sets were observed. These results are compatible either to a tetragametic chimera or to the fertilization of the second polar body after 8p crossing-over. The strategies and difficulties to study mechanisms leading to the production of chimeras will be discussed.

P0442. Prenatal diagnosis of trisomy 17 mosaicism identified in amniotic fluid cell cultures. Case report

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We have found 10 cases of trisomy 17 mosaicism detected in amniotic

fluid reported in the literature. 9 of them had resulted in normal offspring with no postnatal evidence of trisomy 17 cells. The 10th case was terminated and pathological examination showed intra-uterine growth restriction and some minor dysmorphism. Complete trisomy 17 has never been reported in a live birth.
Our patient 33 years old pregnant woman, healthy, no bad habits. This was her 1st pregnancy, expected. She consulted State Medical Genetic Clinic, Prenatal Diagnostic Unit due to concerns about pregnancy in her age. Biochemical results: AFP 24.23 Sv/ml (25.5-50.9) (AFP MOM 0.68), free βHGT 29.85Sv/ml (5.3-15.8) (free βHGT MOM 2.32), ARISK 1:574, DRISK 1:134. During pregnancy twice (15th and 24th week) ultrasound investigation was done - no pathology of fetus was found. Due to results of biochemical analysis diagnostic amniocentesis was performed in the 16/17th week of pregnancy. Karyotype 47,XX+17[2]/46,XX[18].
The baby was born on 17 Nov, 2005, weight 3620g, length 55cm, Apgar score 8/9, without any phenotypic pathology. Neurosonography, echocardiography, chest X-ray showed no clues of disturbed development. Fundus oculi without pathology. Abdominal ultrasonography revealed dystopy of the left kidney (located behind bladder), hydronephrosis. Karyotype analysis was repeated in peripheral blood - 46,XX. Unfortunately we have no possibility to check for the karyotype in fibroblast culture.
As a result this case of prenatal trisomy 17 mosaicism resolved in normal kariotype. It still remains a question of discussion if the kidney ectopy would be associated with the chromosomal abnormality.

P0443. Genetic screening of embryos and foetuses. Should we restrict the tests available and how could that be achieved by law?

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The community might decide that it is socially or morally desirable to prevent women trying to “choose” the child they want, either by pre-implantation genetic tests; or by a prenatal test followed by termination of pregnancy (TOP) if the foetus has a genetic abnormality. But would the law be effective in implementing such a policy?
A law that prevented TOP for relatively minor foetal abnormalities would seem inconsistent with other grounds on which pregnancy may currently be lawfully terminated, in practice if not according to the strict letter of the law.
Alternatively, a new law that penalised the conduct of genetic tests except for certain “serious” conditions would be difficult to draft. What conditions would be included? One might state the prohibited conditions in broad terms, such as those that involve an essentially “cosmetic” condition like cleft lip, rather than a medical one. Or, having agreed on broad principles to be included in legislation, a committee could be established to determine periodically the conditions to be included. That approach would have the benefit of flexibility - the conditions for which testing is prohibited could be changed fairly simply as new tests become available. However, the decisions would be made without public knowledge and without having to seek legislative amendments, which seems undesirable, especially in such a contentious area.
This paper discusses and assesses these legal options for regulation.

P0444. Detection of toxoplasma infection from amniotic fluid samples with quantitative real-time PCR method

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Background: The postnatal infection caused by the intracellular parasite *Toxoplasma gondii (T. gondii)* is often asymptomatic or has mild symptoms. The prenatal toxoplasma infection can cause serious congenital malformations in the affected fetuses. As serological methods can not detect directly the active fetal infection, the analysis of the amniotic fluid with PCR based methods is essential for the diagnosis of intrauterine toxoplasma infection. According to the literature quantitative real-time PCR is the most sensitive and reliable method of the molecular biologic methods. We decided to introduce and to test the method with our prenatal samples.
Methods: We tested 70 amniotic fluid samples obtained from women showing seroconversion during pregnancy for the presence of *T. gondii* using quantitative real-time PCR. Primer-probe design and the PCR conditions were used according to Contini et al.(Int J Parasitol 2005).
Results: The real-time PCR using fluorescence energy transfer hybridization probes has the sensitivity to detect one parasite in the sample. We detected 6 *T. gondii* positive samples from the studied 70 amniotic fluid samples.
Conclusions: The quantitative real-time PCR based methods is a sensitive, simple and easy to perform method for the prenatal diagnosis of intrauterine toxoplasma infection.

P0445. Cytogenetic analysis of cystic hygroma detected at prenatal diagnosis: a report of 16 cases

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Cystic hygroma is an anomaly of the vascular lymphatic system, which is commonly, saw in the neck region. It occurs at a rate of 1:6 000 pregnancies.
In approximately 60% of cases, it is associated with an abnormal fetal karyotype, including Turner syndrome, trisomies 13, 18 and 21.
During July 1998 to December 2005, sixteen cases of cystic hygroma were studied in our Prenatal Diagnosis Center.
Maternal ages at the time of the diagnosis ranged from 20 to 42 years. Amniocentesis was performed between 13 and 23 week’s’ gestation and chromosomal abnormalities were diagnosed in 62% of the pregnancies. Turner’s syndrome was found in 3 cases and trisomies in 5 (three trisomy 21, one trisomy 13 and one trisomy 18).
Despite the number of cases study were small the results obtained were like the previous reports: the majority of cases (62%) had abnormal karyotype and in 80% were aneuploidies.

P0446. Fluorescent probe detection of *deltaF508del* cystic fibrosis allele in different tissues.

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Background: Cystic fibrosis (CF) is the most common autosomal recessive genetic disorder in the Caucasian population. The molecular diagnosis is difficult since there are about 1000 mutations in the cystic fibrosis transmembrane regulator gene (*CFTR*). 64% of the CF cases have *ΔF508del* in Hungary. Determination of *ΔF508del* is one of the most commonly performed genetic analysis in prenatal diagnostic centers in Central Europe.
Method: 121 DNA samples isolated (using High Pure PCR Template Isolation kit, Roche, Germany) from different tissues (87 blood samples, 18 chorionic villus samples and 14 amniotic fluid samples) were involved in the study. Quantitative real-time PCR with melting curve analysis was performed to detect *ΔF508del* (LightCycler FastStart DNA Master Hybridization Probes kit, Roche). The primer-probe set and the PCR conditions were used according to Gundry et al (Gene Test 3;365-370,1999).
Results: 69 healthy normal samples, 44 heterozygous samples, 6 *ΔF508del* homozygous samples and two *ΔF508C* homozygous samples were detected by quantitative real-time PCR combined with melting curve analysis
Conclusions: The quantitative real-time PCR with melting curve analysis is a reliable and fast method for the detection of *ΔF508del*. The results are ready in one hour following the DNA isolation. The applied primer-probe set with melting curve analysis gives additional information for the presence of other mutations in the *ΔF508del* region.

P0447. Prenatal Screening of Down’s Syndrome in Estonia (1995-2004).

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Basic statistics. Area: 45214 km^2. Population: 1,46 million. Birth rate in 2004: 13 868 newborns. Maternal age at delivery > 35….11,4 % (1580 women). Since1995-1998 screening was done for advanced maternal age only, since1999-2004 screening for advanced maternal age and maternal serumscreening (women < 35).

Screening for advanced maternal age (> 35). During the whole period when prenatal diagnosis has been used (1995-2004) 64 cases (63,4%) SD of advanced maternal age risk group have been diagnosed prenatally. During the last three years 78% of the SD cases have been detected. In 2004 , 52% of women > 35 had fetal karyotypes.
Maternal serum screening (double test) started in autumn 1998 in Tartu and only in 2002 in the whole of Estonia. In 2004, 84% of pregnant women in Estonia were monitored. The proportion of pregnancies screened varies: 29% in Eastern Estonia, 90-95% in Tartu and Tallinn, the two biggest cities in Estonia. Over the period of six years (1999-2004) 17 cases of DS have been diagnosed prenatally. During this period, only 19,3% of DS born women < 35 were diagnosed prenatally
Conclusion. During 15 years (1995-2004) when PND has been used in Estonia, 37,6% of DS were diagnosed prenatally. During the last two years 62% of all DS cases have been detected prenatally
Incidence of Down Syndrome in Estonia after prenatal screening was started has decreased: provisional incidence of DS in Estonia is 1: 646 and after prenatal screening (1995-2004) the incidence of DS is 1: 945 in live birth.

P0448. The first and the second trimester Down syndrome screening in Saint-Petersburg

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Total screening for Down's syndrome (DS) and neural tube defects has been used as a double test (AFP & total HCG) since 1997. More than 85 % of all pregnancies were tested per year since 2000. Over 190000 pregnancies were tested in the second trimester (test systems "Alkor-Bio", Saint-Petersburg). Since 2000, homemade software was accomplished and applied for risk calculation ("Medinformatika", Saint-Petersburg). Combined first trimester screening is launched since 2003 . It includes measurements of nuchal translucency (NT), nasal bone, serum markers (PAPP-A and free beta-HCG) . Efficiency of others US markers such as maxillary, femur and humerus lengths and thickness is under inspection. Biochemical screening in the first trimester is performed with Wallac equipment, risk calculation is made by Life Cycle software. Serum of more than 2000 patients was investigated. Detection rate with cut off 1:250 was 88.9% in the 1 trimester (16/18 cases of DS). False positive rate (FPR) was 9.1% in routine screening and was about 4.7% in patients younger than 35. Altogether 31 of 40 fetuses with different chromosomal aberrations were revealed by 1st trimester biochemical screening. Since 1997 186 cases of DS were detected on 15 - 22 weeks of pregnancy. Detection rate in the second trimester with cut off 1:360 was 74.2 % with FPR about 6.8 %.

P0449. Prenatal serum screening for fetal Down syndrome

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We conducted study levels of serum markers used in screening for Down's syndrome, using PAPP-A, AFP, β-hCG measurements. Combined risk Down's syndrome of fruit estimated of the levels serum markers by the Life Cycle program (Wallac, Finland), in view of age and weight of the women. The term of pregnancy had an estimate gestational age based on 'dates' (time since the fist day of the last menstrual period) and an estimate based on an ultrasound scan examination. The risk of Down's syndrome was - 1:220.
We have screened 839 pregnant women for Down's syndrome (I trimester - 197, II trimester - 642). In the first trimester of a deviation in biochemical markers are revealed at 36 women, in second trimester - at 94 pregnant. In the risk group for chromosome pathology was included 87 pregnant. The invasion diagnostics have revealed 10 pregnant women with the chromosome pathology: 5 - trisomy 21; 1 - trisomy 18; 3 - structural chromosome aberration. Is marked, that from these 10 pregnant at 3 pregnant women had deviations serum of markers were combined with ultrasonic markers, at 4 pregnant women - with the age factor. The median levels for Down's syndrome were PAPP-A -1,4 MoM, AFP - 1,3 MoM, β-hCG -1,96 MoM, at norm from 0,5 up to 2,0 MoM.
The preliminary results of screening for Down's syndrome show low efficiency based only on levels of the serum's markers. We have found out higher sensitivity β-hCG in comparison with PAPP-A, AFP. Highly efficiency of the screening at estimate of the combined risk for Down's syndrome was reached by the serum markers in a combination with the data of ultrasound scan determination.

P0450. Assessment of fetal sexing and RhD genotyping using cell free fetal DNA in maternal plasma on clinical samples

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Background: The finding of cell free fetal DNA (ffDNA) circulating in maternal blood has opened new applications in non-invasive prenatal diagnosis. Measurable amounts of ffDNA are present in maternal circulation and increase throughout pregnancy. This makes possible detecting paternally inherited sequences such as RhD and SRY. Real-time PCR detection of fetal DNA in plasma is easy to automate allowing high throughput that simplify its large scale clinical application.
We aimed at developing a multiplex assay for detection of both genes in a single reaction evaluating its sensitivity and specificity on clinical samples.
Methods: Blood samples were collected from 45 RhD negative women between 13 and 28 weeks of gestation (mean 14w). Four different DNA extraction procedures were evaluated on few selected cases before all samples were processed.
Real-time PCR assay was developed to amplify RhD and SRY sequences using differently labelled Taqman probes. Results were than compared with those obtained on newborns.
Results: SRY sequence were detected in 25 plasma samples, RhD was detected in a total of 35 fetuses (22 males and 13 females). The absence of both products in 7 cases was considered evidence of female RhD- fetuses.
Newborn analysis confirmed all results without false positive or false negatives.
Conclusion: Real Time PCR detection of RhD gene has proved to be efficient and reliable allowing prompt identification of RhD+ fetuses without false negative results. Simultaneous assessment of fetal sex was also possible in all cases. The procedure proved to be sensitive enough to be applied on clinical cases.

P0451. Fetal hydrops: a retrospective survey over a 5-years time period.

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Fetal hydrops is a serious complication during pregnancy, with an incidence of ca. 1 in 3000. Since antibody screening against blood groups has been introduced on a routine basis, most cases are due to other causes than blood group antagonism and belong to the group of non-immunological hydrops foetalis (NIFH). A wide variety of causes are held responsible in the latter such as cardiac abnormalities, chromosomal aberrations, twin to twin transfusion syndtrome (TTTS), infections, monogenic and metabolic diseases, anemia, anatomical abnormalities of various organ systems, and skeletal dysplasias. Detection of fetal hydrops during pregnancy should be followed by standardized procedures to obtain a diagnosis which is essential for accurate counselling of the parents. Therefor, we reviewed experiences in our "Perinatal Group", Academic Hospital Maastricht, The Netherlands, during a 5-years time period. This was before the introduction of the first trimester screening in combination with nuchal translucency measurements. Our observations in 61 cases are reported and a protocol for multidisciplinary use is proposed in the case that a fetal hydrops is detected.

P0452. Prenatal diagnosis of t(7;10)(p22;p12.1) inherithed from mother

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A 32 year old Caucasian female pregnant for the forth time was referred for genetic counselling at 18 gestational weeks. She presented the following family history: two female children from the first and third pregnancy with mental retardation and the second pregnancy ended with a stillborn male fetus (with no karyotype). The karyotype for neither one of the members of the family had been previously established. The ultrasonographic examination of the fetus was normal. The cytogenetic analysis with GTG banding of amniotic fluid cells revealed the existence of a balanced chromosomal translocation: 46,XX,der(7),der(10),t(7;10)(p22;p12.1). Peripheral blood samples were taken from the mother, father, and second child for karyotyping, in order to establish the translocation producing mechanism, "de novo" or inherited. The father's karyotype was found to be normal, the mother had a balanced rearrangement involving the same region of these chromosomes, and the second child presented partial 7p trisomy. As a result, we considered the unborn child's karyotype anomaly to be inherited from the mother. After extensive genetic counselling, the parents decide to keep the pregnancy. At the present time, this pregnancy is not finished.
Conclusions: The fetal karyotype granted us with the possibility to take the correct decision involving this pregnancy. These tests allowed us to determine the etiology of the mental retardation of the second child as well.

P0453. Multiple fetal malformations in diabetic pregnancy

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Background: Diabetes during pregnancy is associated with a high risk state for both mother and her fetus. Spontaneous abortion, preterm labor, preeclampsia, birth injury, macrosomia, birth defects and future metabolic abnormalities are more common in pregnant diabetics. The pregnancy outcomes in diabetic women are related to glycemic control during pregnancy. Identification and careful management of diabetes during pregnancy can avoid or minimize the complications associated with diabetic pregnancy. **Objectives**: To describe and analyze the severity of congenital malformations associated with diabetic pregnancy. **Patients and Methods:** A 23-year-old pregnant diabetic female was referred at 18 weeks' gestation for a routine prenatal ultrasound. Fetal monitoring was made by ultrasound scans for fetal growth, congenital malformations, and amniotic fluid volume. We also collected information about family medical history. Amniotic fluid samples were taken to perform prenatal cytogenetic diagnosis. **Results**: Ultrasound examination revealed a singleton pregnancy with multiple congenital malformations: craniofacial defects, cardiac defects, gastrointestinal defects, renal agenesis, undescended testis and anomalies of the limbs. Many of the same malformations are also seen in trisomy 13 but karyotype analysis indicated a normal cytogenetic male: 46, xy. The pregnancy was terminated at 28 weeks of gestation. Autopsy findings confirmed the ultrasound diagnosis. Postpartum radiologic examination showed missing ribs and absent lumbosacral vertebrae. **Conclusions**: unplanned pregnancy and inadequate glycemic control of diabetic women before and during pregnancy increase the risk of congenital malformations.

P0454. Absence of nasal bone in fetuses with trisomy 21 and other chromosmal abnormalities at 11-14 weeks of gestation: a retrospective study

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Several studies have demonstrated a high association between absent nasal bone (NB) at the 11-14 wk scan and trisomy 21. Evaluation of the presence or absence of the NB for screening for trisomy 21 was retrospectively assessed in 181 pregnant women at 11-14 wk gestation.
Of the 181 pregnant women that underwent invasive test, 161 had normal karyotype. Of the 20 chromosomal abnormalities, trisomy 21 was in 11 cases, trisomy 18 was in 6 cases, and in 3 cases other abnormalities were found.
In the whole study group (n=181) the NB was present in 123 (68%), absent in 12 (7%) and was uncertain in 46 (25%) cases, while in the 161 cases with normal karyotype the NB was present in 119 (74%) absent in 4 (2%) and uncertain in 38 (24%) cases.
In trisomy 21 (n=11) the NB was absent in 5 cases (46%), present in 3 (27%) and was uncertain in 3 (27%). This gives a sensitivity of 63% and a specificity of 95% and false positive rate of 5.6 for the absence of NB in trisomy 21.
In remaining 9 chromosomal abnormalities the NB was present in 1 case absent in 3 and uncertain in 5 cases. This gives a sensitivity of 67% and a specificity of 98% and false positive rate of 3% for the absence of NB in other chromosomal abnormalities. Absence of the NB is an important marker of trisomy 21. However, an appropriate training of operators is mandatory.

P0455. Free foetal DNA detection in maternal plasma using STR loci.

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Background: The foetal genotype recognition in maternal plasma in pregnant women is solved mostly by real-time systems. In this case the Y- chromosome specific probes from male specific region (MSY) are used to distinguish male fetus. This work supported by IGA MZCR NR 7817-3 describes possibilities in free foetal DNA detection and quantification by STR.
Methods and Results: Artificial genotype mixtures ranging from 0,2 % to 100 % to simulate maternal and paternal genotypes and 27 DNA samples from pregnant women in different stage of pregnancy were used for DNA quantification and detection. Foetal genotype was confirmed by biological father genotyping. The detection was performed in STR from 21st chromosome Down syndrome (DS) responsible region by innovated refined (R) QF PCR which allows to reveal and quantify even very rare DNA mosaics.
The STR quantification was assessed in artificial mixtures of genotypes and discriminability of particular genotypes was on the few percent level. Foetal DNA was detected in 74 % of tested samples.
Conclusions: The RQF PCR application in quantification and differentiation between maternal and foetal genotypes by STR loci could have importance in noninvasive prenatal diagnostics as another possible marker for DS risk assessment.

P0456. Molecular and expression analysis extends the phenotypic spectrum of *GLI3* mutations to agnathia and oligosyndactyly

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GLI3 mutations result in Greig cephalopolysyndactyly (GCPS), Pallister-Hall (PHS) and isolated polydactyly (PD). PHS first described as a lethal condition associates hypothalamic hamartoblastoma (HH), post axial PD and imperforate anus (IA).GCPS combines PD and craniofacial features. Recently, phenotype-genotype correlation have been shown for both the location and the nature of *GLI3* mutations.
Here we report on a molecular study of *GLI3* in 18 patients and 23 fetuses selected for i) HH and PD (10), ii) IA plus 2/5 features of the PHS spectrum (13) i.e. growth retardation (IUGR), micropenis, limb, heart, or renal anomalies, most were VACTERL cases.
We identified a heterozygous *GLI3* mutation in 12 cases (7 GCPS, 4 PHS, 1 PD). In all GCPS cases with corpus callosum anomaly in our series (3) and previous reports (3), the truncating mutation was in the C terminal domain of the protein suggesting a further genotype-phenotype correlation. In one fetus, the association of IUGR, PD, renal agenesis without IA led first to the suspicion of Smith-Lemli-Opitz. Another fetus had a severe and unusual phenotype including agnathia, absence of oral orifice and oligosyndactyly of 4 limbs. *In situ* hybridisation confirmed *GLI3* expression in human pharyngeal arches then mandible. No mutation was identified in cases with HH and PD as part of another syndrome or in VACTERL cases.

All mutations but one were novel and consistent with the genotype-phenotype correlation. Our results emphasise on the possible lethality of *GLI3* mutations and extend the PHS spectrum to severe craniofacial and reductional limb defects.

P0457. Prenatal diagnosis in late-onset neurological disorders in Portugal: experience with 83 requests, since 1996

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PND in adulthood-onset disorders presents several specific issues, namely the possibility of several decades of a (physically) healthy life and the need for the decision-making process regarding termination of pregnancy (TOP) to be completed before disclosure of the results, to avoid performing a presymptomatic test (PST) in an unborn child for an incurable disease (precluded by ethical and, sometimes, legal reasons).

Since 1996, we received 83 requests for PND, in familial amyloid polyneuropathy (FAP-I) ATTRV30M (53), Huntington disease (11), Machado-Joseph disease (4), SCA2 (2), Friedreich ataxia (11), and others (2).

PND was requested for the first pregnancy of 32 couples, while 20 already had one or more children; 5 couples had 2 requests, and one had 3. The affected/carrier/at-risk parent gender ratio was 1,2F/1M. Only 5 inquired about PND before actual pregnancy; about 2/3 requested PND before 12W gestation (mean=10.2W). Only 34 invasive procedures (15 amniocenteses and 19 chorionic villous samplings) were actually performed (often, parents at-risk, tested during pregnancy, were 'non-carriers').

About 1/3 of cases were simultaneous requests for PST, a rather stressful situation, given time constraints, and the potential for three distressful events in a short period (PST, PND, TOP). Of the 20 'carrier' foetuses, only 19 underwent TOP. One pregnancy was not terminated despite a 'carrier' result, due to a late reversal of the couple's decision; non-termination of this foetus implied the birth of a child whose parents know will become affected.

PND in late-onset diseases demands tactful counselling, acute sensibility and intensive psychosocial evaluation and support.

P0458. Femoral incurvation and intra uterine growth retardation : which diagnosis ?

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When femoral incurvation and intra uterine growth retardation are diagnosed antenatally, finding the proper aetiology is difficult and leads to various hypothesis. We report on a bi- chorial, bi-amniotic twin pregnancy, during which a «short femur» was noticed at 26 WG on one of the twin. Fetal chromosomes were normal, maternal infection was ruled out, and fetal intrauterine x-rays confirmed femoral incurvation and osteoporosis. At birth, at 37 WG, intra uterine growth retardation and rhizomelic shortening of the limbs were present. Skeletal x-rays, performed at 3 days of life, revealed osteoporosis, enlarged irregular metaphyses of the long bones, and bilateral femoral incurvation. Assessment of phosphorus and calcium metabolism showed severe hypercalcemia and increased alkaline phosphates. Parathyroid hormone was at the upper limit of normal. There were no clinical sign of hypercalcemia and no lithiasis was identified. Treatment by bisphosphonate was started, allowing a rapid decrease of the calcemia. Since hyperparathyroidism was suggested, a molecular analysis of the Calcium Sensor Receptor gene was performed, which revealed a heterozygote mutation responsible for the synthesis of a truncated protein. This mutation had been previously reported in cases of familial hypocalciuric hypercalcemia, and, when associated with another mutation, in one case of severe neonatal hyperparathyroidism. Molecular analysis in the parents and the other twin are pending. Reviewing the literature, severe neonatal hyperparathyroidism can also be secondary to maternal hypoparathyroidism or type II mucolipidosis. We discuss the management of femoral incurvation during pregnancy and the clinical evolution of this case after 6 months of treatment.

P0459. Chromosome abnormalities identified in the population of Eastern Ontario (Canada) found to be at increased risk for Down syndrome by integrated prenatal screening

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Objective: To evaluate the chromosomal abnormalities detected in addition to Down syndrome by amniocentesis through referrals based on integrated prenatal screening (IPS) in the Ottawa-Gatineau region of Ontario.

Methods: In the first trimester, women had an ultrasound (between 11 and 13+6 weeks of pregnancy) to obtain a nuchal translucency measurement and provided a blood sample to determine PAPP-A levels. In the second trimester, a second blood sample was drawn at 15+3 weeks until 18+6 weeks of pregnancy to measure biochemical markers AFP, estriol and hCG. The results of the nuchal translucency and the four biochemical markers were combined to calculate a risk for Down syndrome for the second trimester. Women identified to be screen positive (≥1/200 term risk) were managed according to standard practice.

Results: Between July 2002 and December 2005, 19 175 women provided a first and second trimester sample. The total number of IPS screen positive cases for Down syndrome was 645 (3.36%) and for trisomy 18 was 55 (0.3%). Amniocentesis was performed in 488 (73.6%) cases. The abnormalities detected include the following: Down syndrome (6.6%), trisomy 18 (1.0%), trisomy 13 (0.6%), other autosomal trisomies (0.2%), triploidy (0.4%) and sex chromosome aneuploidy (0.6%). Other abnormalities included inherited balanced rearrangements (0.8%), de novo unbalanced rearrangements (0.4%) and sex chromosome mosaicism (0.2%).

Conclusions: IPS has been successfully implemented in the Eastern Ontario region. The study described herein shows that other chromosomal abnormalities are also detected. Taken together, these findings are important considerations when offering IPS as a screening strategy.

P0460. The meaning of informed consent for congenital anomalies after IVF

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Every year more and more couples are engaged in vitro fertilization all over the world. But recent scientific publications (American Journal of Human Genetics, 2004,Vol 75., Congenital anomalies, 2005,Vol 45) suggest that there is an elevated risk of imprinting defects and syndromes connected with IVF e.g. Wiedemann-Beckwith, Angelman, Prader - Willy. In Lithuanian Centre for Medical Genetics we have observed several congenital anomalies (including fatal) in children conceived after IVF. There is no register of how many IVF procedures are performed every year, because these procedures have been performed only in private clinics for few years. Approximately there may be 300 children born after IVF in Lithuania.

Only part of woman after IVF, were consulted by clinical geneticists in Centre for Medical Genetics. We report six cases of anomalies after IVF, diagnosed prenatally. Diagnosis of fetuses were: holoprosencephaly, egzencephaly, hydrocephaly, hydrops fetus universalis (2 cases), thoracopagus. All these anomalies in general population are not frequent and the rate is sure less than 1 in 300. We have come to the conclusion that it is necessary before IVF procedure to inform the couples about an increasing possibility of having a child with malformations. It would seem that the gametes of people with sterility problems are more likely to develop genetic errors.

P0461. The approach to fetuses with microcephaly in the multidisciplinary fetal neurology clinic

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Microcephaly is defined postnatally as low brain weight and a small head circumference (HC) more than two standard deviations (SD) below the mean or below the 3rd percentile. Such a broad definition obviously includes normal individuals. The smaller the head circumference, the higher the chances of associated mental retardation. The prenatal diagnosis of microcephaly, particularly in cases of primary microcephaly, is usually difficult before the 3rd trimester**.**

The etiologic heterogeneity and variability of microcephaly in genetic syndromes are among the more difficult issues in prenatal ultrasound in pregnancies either with an incidental finding of this anomaly, or in cases with a recurrence risk, thus the counseling for fetuses with a small HC is difficult.

Mental retardation can safely be predicted in cases with associated US findings, abnormal karyotype or positive test for intrauterine infection. In fetuses with isolated small HC an effort should be made to determine gyral normality in utero by US or MRI.

The main tasks of the multidisciplinary clinic are to try to give the most accurate diagnosis and prognosis and to counsel and escort the parents through this time of crisis and crucial decisions. To illustrate these difficulties we will present our experience with 38 fetuses that were referred to our clinic with suspected microcephaly (2001-2004), including neuro-developmental follow-up of these babies.

A flow chart for prenatal investigation of fetuses with microcephaly will be displayed.

P0462. Use of the Multiplex Ligation-dependent Probe Amplification (MLPA) as a complementary technique for the assesment of a structural chromosomal mosaicism in prenatal diagnosis

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The presence of a structural chromosomal mosaicism in prenatal diagnosis is a rare event that may represent a major diagnostic problem since they most often occur during cell culture.

We describe a case of a pregnant woman who underwent an amniocentesis due to maternal age. At that time the fetus had no apparent sonographic markers. The karyotype resulted to be 46,XX (35%)/46,XXr(22)(p11;q13.1)(65%).

Once the presence of the mosaicism was diagnosed, and confirmed by FISH with the use of the Di George probe (QBIO-gene), the couple was suggested a cordocentesis in order to confirm the results.

The cytogenetic analysis of the fetal cord blood confirmed the presence of the mosaicism. The percentage of both cell lines in this tissue was 50%.

In parallel to cord blood culture, MLPA technique with subtelomeric probes was performed in order to test its accuracy in the assesment of terminal deletions/duplications for this kind of diagnosis. To discard the occurrence of the event during cell culture, DNA samples from both the original sample and culture were analysed. The structural chromosomal mosaicism was detected in both samples using MLPA.

The MLPA technique was not able to detect the mosaicism in a DNA sample obtained from the cord blood due to the presence of maternal cell contamination.

The MLPA technique has shown to be a good tool for the assessment of structural chromosomal mosaicism involving the terminal deletion of a chromosome. However, the interpretation of the results has to be done carefully when a cell contamination is suspected.

P0463. Norman Roberts syndrome: further delineation of the phenotype in prenatal life

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Norman Roberts syndrome was firstly described in 1976 in a male infant affected by lissencephaly born to consanguineous parents. In the family other two children, a brother and a sister, showed the same clinical spectrum (Norman et al., 1976). Aside from the neuradiological sign, the condition differed from the other forms of lissencephaly (syndromes and isolated) having specific facial dysmorphisms (sloping forehead, broad and prominent nasal bridge and widely set eyes). The authors hypothized an autosomal recessive inheritance. Subsequently five cases were described. (Iannetti et al, 1993 ; Sergi et al 2000 ; Caksen et al 2004).

We report on three new cases of Norman Roberts syndrome. All cases were suspected prenatally and the diagnosis was performed after termination of pregnancy.

The first case is a female fetus for whom a diagnosis of microcephaly was made at 22nd week of gestational age, while the second and the third case were two female fetuses from a couple of unrelated and healthy parents. Microcephaly was detected at 33rd and 22nd week of gestation respectively.

The combined data from US studies, MNR, autopsy and histological finding allowed us to diagnose Norman Roberts syndrome.

To the best of our knowledge only one other prenatal case has been described (Sergi et al, 2000); together with our cases a better characterization of the prenatal phenotype of the condition can be delineated, completed with a detailed histological study.

P0464. A new osteochondrodysplasia resembling OPD2 in a severely affected female fetus with negative *FLNA* mutation analysis

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Otopalatodigital syndromes (OPD) are allelic X-linked disorders characterized by skeletal abnormalities including hands and feet abnormalities, facial dysmorphism and cleft palate in males, OPD type 2 representing the severe end of the spectrum. We report the first pregnancy of non-consanguineous parents. Ultrasound survey at 12 and 17 WG showed nuchal translucency and severe micromelia with bowing of long bones. Cytogenetic study performed on amniotic fluid revealed a normal female karyotype. Post-mortem study after termination of pregnancy at 19 WG showed severe growth retardation with short and bowed limbs, facial dysmorphism with cleft palate, preaxial polydactyly of both feet, severe hypomineralisation with cranial vault hypocalcification. Although many clinical and radiological features were in favour of OPD2 syndrome, the severity of micromelia, early lethality, the presence of generalized osteopenia instead of hyperostotic bones and the female sex of the foetus with random X-inactivation studies ruled out this diagnosis. In addition, sequencing analysis did not reveal any pathogenetic mutation in the *FLNA* gene. In conclusion, we postulate that this female foetus presents a new entity of the OPD spectrum disorder.

P0465. A severe overgrowth foetal syndrome of unknown origin, detected at 28th week of gestation

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A 37 years old pregnant woman at her third pregnancy, come to our attention for a counselling after several ultrasound examinations at 28th week. A prenatal ultrasound at 13th + week was performed to program drawing of AL for maternal age and showed an increased foetal nuchal translucency (NT=4,1mm). Cytogenetic analysis revealed

normal female karyotype (46XX). Ultrasound examination at 20th week showed a hydronephrosis without ureteral dilatation. Repeated US scan disclosed progressive macrosomy, macrocephaly, shortening of long bones (<5th centile), hepatomegaly, mild left ventricle hypertrophy, bilateral hydronephrosis, redundant skin, course facial features and polyhydramnios. NMR of CNS showed no other malformations except macrocephaly with an increase of liquoral pericerebral spaces.
The pregnancy terminated with intrauterine foetal death at 30th week.
At post-mortem examination, the foetus was macrosomic and macrocephalic for gestational age. Furthermore anatomopathological evaluation revealed craniofacial dysmorphisms (hypertelorism, flat and large nose, macroglossia, thick lips), low-set ears, short neck with redundant skin, thick skin and hydrocephalus. No evidence of omphalocele or diaphragmatic hernia or nephroblastomatosis. Epatomegaly, hypertrophic cardiomiopathy and bilateral hydronephrosis associated with mild bilateral ureteronephrosis were also present. The external genitalia were female and hypertrophic.
Samples were taken to exclude metabolic diseases.
The parents are Caucasian, healthy and non consanguineous. Family history is unremarkable.
The foetus is affected by a constitutive syndromic condition, whose diagnosis is not yet clear.
The cytogenetic and molecular cytogenetic analysis for subtelomeric regions of the parents is in progress. The possible differential diagnoses are here discussed.

P0466. Genetic Indications for Preimplantation Genetic Diagnosis compared with Prenatal Diagnosis

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Prenatal genetic diagnosis technology is widely used to identify the genetic status of an at-risk embryo or foetus. This clinical audit aimed to review and compare the clinical indications and demographic characteristics of couples undergoing prenatal genetic testing by pre-implantation genetic diagnosis (PGD) with a private service and by standard prenatal genetic diagnosis (PND) in a public hospital . In addition we collected information about the efficacy of each technique.
The principle positive finding of this audit was an increased uptake of PGD for predictive testing/adult onset disorders. This was not unexpected, but is of importance for future service provision. In addition we found that the birth rate per PGD cycle in this cohort was 26.4%
This study is a preliminary examination of limited data. Future studies should focus on couples' reasons for seeking PND or PGD services, their religious and cultural beliefs and the financial factors affecting their decisions.

P0467. The evaluation of chromosomal abnormalities diagnosed prenatally in Izmir

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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. Chromosomal abnormalities are observed approximately 0.5% to 1% of infants born with multiple anomalies and cause 4-7% of perinatal deaths. The vast majority of chromosomal abnormalities detected in prenatal samples are trisomy 13,18, 21 and sex chromosome aneuploidies. 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 4438 pregnant cases in Izmir between the period of 1998-2005. The overall chromosomal abnormality rate was found to be 157/4438 (3.53 %). Eighty-eight out of 4438(1.98%) cases had common triosmies which were 1.48% (trisomy 21), 0.29% (trismoy 18) and 0.2% (trismoy 13). The structural chromosomal and sex chromosome abnormality rates were found to be 1.19% and 0.4% respectively. Among referral indications, the proportion of pathological ultrasonographic findings has been significantly increased in recent years. This shows that improvements in the ultrasonographic techniques and ultrasonographic examinations positively effects the prenatal diagnosis of chromosomal abnormalities. Careful genetic counseling by expert geneticists regarding the patients indications is essantial for determining the cost-effective prenatal diagnostic test for each patient.

P0468. Biomedical Research Centre: the first laboratory in Lithuania started to use FISH for prenatal diagnostics

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Biomedical Research Centre was the first one in Lithuania started to perform prenatal diagnosis of aneuploidy by using FISH procedure. FISH (fluorescence in situ hybridization) procedure may be effectively used to perform preliminary screening of numerical abnormalities involving chromosomes 13, 18, 21, X and Y in human cells.
Methods: FISH was performed by using commercial reagent kit *AneuVysion Multicolor DNR Probe Kit* (CEP 18, X Y-alfa satellite, LSI 13 and 21), VYSIS, USA. This kit allows investigating chromosomal abnormalities in very early pregnancy - from 10 weeks.
Results: Patient's amniotic fluid samples have been collected from private clinics of Lithuania. The FISH procedure has been was performed on 10 samples of amniotic fluid (in 10-16 weeks of pregnancy). The chromosomal aneuploidy was detected only in one patient: nuc ish 13q14×2, 18p11.1-q11.1(D18Z1×2), 21q22.13-q22.2(D21S259×2), Xp11.1-q11.1(DX21×2),Yp11.1-q11.1(DYZ3×1). The obtained chromosomal aneuploidy determines Klinefelter syndrome in fetus.
We are very happy to perform early prenatal diagnosis in our laboratory. Women have a possibility to do that examination from 10 week of pregnancy, while standard cytogenetic analysis might be performed only from 14 weeks. Prenatal diagnosis by FISH let to know real situation about fetus health, would vanish worry about coming new child into our world.

P0469. The Influence of Bioethics and Religious Ruling in Prenatal Diagnosis and Abortion

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"Bioethics" means the study of ethical issues arising from human involvement with life. Many concepts of bioethics can be traced in religions and cultural patterns that may or may not share some universal ideals. This is especially true when we talk of reproduction and genetics, prenatal diagnosis, abortion etc.
Decisions to undertake prenatal diagnosis, with more emphasizes on Iran, involve a complex assessment of the best interests of the fetus and parent's interest. Parents often accept a recommendation for prenatal diagnosis that is approached collaboratively, especially if the test is of proven efficiency and have a low maternal/fetus risk and also low cost. The prenatal diagnosis poses a potential conflict between the parent's own best interests, ideology, and very importantly faith on one side and perception of the best interests of the fetus. In addition, if prenatal diagnosis is linked to selective abortion then the autonomy of the fetus plus religious faith of the parents could lead to a conflict.
From the prenatal diagnosis experiences in Iran several other concerns have also been noticed by geneticists. These concerns were as follows: fairness of access to genetic services, indications for prenatal diagnosis, confidentiality problems, counseling incapacitated persons etc. One of the extremely important items to be concerned for the parents found to be abortion choices and legal restrictions due to Islamic faith and Islamic low practicing in Iran. Considering these factors, a code of ethics practical in Islamic countries such as Iran seems very necessary to be proposed

P0470. Bioethical problems of fertility awareness and prenatal diagnostics

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Postponing childbirth is becoming increasingly common in Western countries, especially among groups with higher education qualifications. This increases the risk of involuntary infertility in this group and reproductive problems connected with age. In November 2005 European Commission publicized results of two surveys on science and technology and their related values. One of the issues examined was prenatal life. 53% of Europeans think it is “very important” to protect the dignity of the foetus. The countries most concentrated on this issue (73%) are Malta, Greece and Ireland. Hungary, Denmark and Lithuania are at the bottom of the list (only 37%). These attitudes are important from the medical point of view, because every elective abortion has aggravating effect for future pregnancies. After abortion future pregnancies appears to be in elderly age, when there is elevated possibility to chromosomal defects. Also there are risks for preterm birth and delivery complications.

An important aspect of prenatal diagnostics (PD) is to discuss with a couple about advantages and disadvantages of PD.

We report some cases:

Case1.36 aged woman came to genetic counseling clinic with fertility problems: in anamnesis second and third pregnancies ended in miscarriages at 6-7 weeks, after first pregnancy ended in miscarriage at 17 weeks, after amniocentesis (answer of karyotype was normal 46XY).

Case2. 42 aged woman, who previouslly had 6 abortions, delivered child with Patau syndrome.

Case3. 37 aged woman, who conceived and used oral contraceptives, not realising about pregnancy, till third month of pregnancy, came for PD.

P0471. Weight and ethnic adjustment factors for Chinese women undertaking prenatal screening for Down syndrome

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Ethnic differences in the concentrations of maternal serum markers have been reported in previous studies. The major differences were found in Afro-Caribbean and South Asian women. We conducted a study to investigate the levels of serum markers and to estimate ethnic and weight correction factors in Chinese women. Our study population consisted of 400,650 Caucasian and 30,960 Chinese women who were non-diabetic, had an unaffected singleton pregnancy and who were screened in the Ontario Multiple Marker Screening Program between 10/1993 and 8/2004. Chinese women were identified from the ethnicity category of Asian using a published Chinese surname list. The median maternal weight was 66.3 kg for Caucasian and 54.5 kg for Chinese. Using the current weight correction factors derived from Caucasian women, the median MoMs of AFP, uE3, total hCG and PAPP-A were 3%, 14%, 19% and 16% higher in Chinese women. The increase in uE3 and total hCG in Chinese women were consistent across most of the weight groups. The increase in PAPP-A was largely due to inadequate weight correction for Chinese women. Quadratic weight correction formulas based on analyte levels in Chinese women were derived, allowing correction for ethnicity and weight. Ethnic correction will have variable effect on the false positive rate (FPR) for Down syndrome for Chinese women depending upon marker combinations as the influence of hCG is offset by the influence of uE3, AFP and PAPP-A. As nuchal translucency is probably increased in Chinese women, this marker correction is expected to reduce the FPR when included.

P0472. The amniotic fluid proteome in pregnancies with Down syndrome

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Proteomic analysis is widely used for the detection of diagnostic markers and the identification of potential drug targets. In the present study 6 amniotic fluid supernatants (AFS) from pregnancies with Down Syndrome (DS) fetuses and 12 from chromosomally normal fetuses in the 17th week of gestation were analysed by two-dimensional electrophoresis. Gel comparison revealed significant differences in the two groups. Spots with different expression levels were excised and proteins identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry and tandem mass spectrometry. Splicing factor arginine/serine-rich 4 (Q08170) was present only in AFS from DS fetuses and completely absent in the control group. Quantitative differences were detected for AMBP (P02760), CO1A1 (P02452), CO3A1 (P02461), CO5A1 (P20908) and PGBM (P98160). These proteins were increased in cases with DS, while protein IBP1 (P08833) was decreased by 40% compared to chromosomally normal fetuses. Four proteins (CO1A1, CO3A1, CO5A1 and PGBM) appeared as fragments.

Our data suggest the feasibility of proteomic approaches in identifying proteins differentially expressed in AFS obtained from cases where the fetus has trisomy 21 and allow further research to be conducted in the area. Since differentially expressed proteins and small peptides are likely to cross the placenta barrier and be detected in maternal serum, proteomic analysis carries the potential to enhance research in the study of noninvasive techniques for prenatal diagnosis of aneuploidies. However, for protein biomarkers to be of any clinical utilit, extensive validation in human trials is essential..

P0473. Prenatal diagnosis in Turkish pregnants on chromosomes 21, 18, 13, and XY with quantitative fluorescent PCR methods

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Quantitative Fluoresent-Polymerase Chain Reaction (QF-PCR) method gives rapid prenatal diagnosis for chromosomal aneuploidies. Common chromosomal aneuploidies, 21, 18, 13, and XY, in 262 Turkish prenatal cases at risk were evaluated. We tested four Short Tandem Repeats (STR) sequences for the chromosomes 21, 18, 13 and five for sex chromosomes in this study. Multiplex PCR assays based on amplification of STR sequences which are tetra nucleotides by using flourescent primers, and visualization and quantification of STR peaks by using automated DNA sequencer with a software were carried out. All findings obtained from QF-PCR method were confirmed with traditional karyotyping analysis. As a result, detemining of common trisomy abnormalities with QF-PCR methods has a great impact for rapid, accuracy, and simple prenatal diagnosis.

P0474. Rapid prenatal diagnosis of common aneuploidies in a amniotic fluid QF-PCR

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The quantitative fluorescent PCR (QF-PCR) has successfully been used for prenatal diagnosis of common chromosomal aneuploidies in the last few years. This method allows us to diagnose common aneuploidies in a short space at time after sampling combined with a high throughput, very low error rates and low cost. QF-PCR technique is based upon the amplification of fetal DNA extracted from uncultured amniotic fluid cells using fluorescent labeled primers. Size separation and allele peak measurements are performed on a semi-automated genetic analyzer. In this study 400 amniotic fluid samples, 3 CVS and 3 cord blood were analyzed for trisomies 13, 18, 21 and sex chromosome aneuploidies using QF-PCR. Test results were compared with those obtained by conventional cytogenetic analysis.

Six cases of trisomy 21 (1,5%), 1 case of trisomy 13 (0,25%), 1 case of Trisomy 18 (0,25%), 1 case of Turner Syndrome (0,25%), 1 case of Klinefelter’s syndrome (0,25%), and 1 case of triploidy (0,25%), 1 case of XXX (0,25%) were detected by QF-PCR. QF-PCR results were consistent with the results of cytogenetic analysis.

This is the first study in which QF-PCR has been used to diagnose the

chromosomal aneuploidies prenatally in a large series of fetal samples in the Turkish population. It is considered that a QF-PCR method is an appropriate choice for rapid aneuploidy testing in our population as has been reported in other populations.

P0475. QF-PCR testing for trisomy 21, trisomy 18 and trisomy 13 on 1300 prenatal samples

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We have evaluated the applicability of 10 STR markers on chromosomes 13, 18 and 21 for prenatal diagnosis of the common autosomal aneuploidies. DNA samples (n=1300) were extracted from amniotic fluid cells, chorionic villi and fetal tissue after abortions. PCR amplifications of 4 markers located on chromosome 21 (D21S11, D21S1411, D21S1440, D21S1435), 3 - on chromosome 18 (D18S535, D18S51, D18S858) and 3 - on chromosome 13 (D13S631, D13S258, D13S256) were performed in four multiplex assays in a combination with 4 markers on the sex chromosomes.
In 235 prenatal samples only QF-PCR analysis was performed; other samples were analyzed both by QF-PCR and conventional cytogenetics - in 73 only DNA diagnosis was available because of culture failures. All samples with trisomy 21 (n=32), trisomy 18 (n=9), trisomy 13 (n=3) and triploidy (n=1) were correctly detected with QF-PCR. For exclusion of maternal contamination and submicroscopic duplication DNA from parents were analyzed when necessary. A polymorphic duplication was detected in one prenatal sample and maternal origin was revealed.
Our experience showed that QF-PCR technique could be used for detection of certain chromosomal numerical anomalies, especially when ultrasound or biochemical analyses are abnormal, or culture failures arise, or cytogenetic analysis is impossible. However the use of this analysis as the only method of diagnosis should be estimated considering the advantages of the technique together with its limitations.

P0476. Preimplantation genetic diagnosis in two couples with balances reciprocal translocations

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Pre implantation genetic diagnosis is a technique based on the transfer process of only those embryos tested to be normal. In this study we aimed to transfer the balanced embryos by means of IVF-PGD in two couples carrying balanced reciprocal translocations of 46,XY,t(12;17)(p11;q11) and 46,XX,t(6;11)(q15;q22). The oocytes were fertilized by means of intra cytoplasmic sperm injection. The resulting embryos were biopsied 3 days after fertilization and the blastomeres were analyzed with fluorescent in-situ hybridization (FISH) where telomeric and centromeric probes were utilized. Balanced and unbalanced embryos were evaluated in two cases. In the first case, 6 out of the 9 embryos (15 blastomeres) analyzed, revealed unbalanced constitution. Only one of the remaining three that revealed balanced constitution was transferred. In second case, some 3 out of the 5 embryos (7 blastomeres) analyzed, revealed unbalanced constitution and one was not conclusive. Another embryo with balanced constitution was transferred. The PGD results were confirmed by amniocentesis in both cases, which resulted in uncomplicated birth of healthy babies, one being a baby boy, the other girl, both with balanced (normal) karyotypes. As a result, we conclude that, in cases identified as translocation carrier, resorting to PGD-supported IVF cycle will reduce down the abortion risk and has a key role in enabling the delivery for healthy babies.

P0477. Prenatal diagnosis of 15q26.1◊qter deletion due to a ring chromosome 15.

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Terminal deletions of chromosome 15q are rare events. Here we report a now case of prenatal diagnosis of 15q26.1◊qter deletion due to a de novo ring chromosome 15. Amniocentesis and foetal karyotyping was performed because of the discovery of oligoamnios, intauterine growth retardation, bilateral plykistic kidneys and diaphragmatic hernia on ultrasound examination at 18 weeks of pregnancy. Conventional cytogenetic study detects a ring chromosome 15 in all analysed foetal cells. FISH was performed for detailed characterisation of this chromosomal anomaly. Whole chromosomic painting, centromeric and locus specific probes of chromosome 15 confirm that rearranged chromosome was a ring chromosome 15 inducing a pure partial monoasomy 15q26.1◊qter
Diaphragmatic hernia and intrauterine growth retardation and kidneys malformations are frequently described in cases of different 15 chromosome deletions; this study contributes to a better delineation of the critical region implicated in these developmental anomalies.

P0478. Molecular diagnosis of Russell-Silver syndrome in a newborn with asymmetric prenatal growth restricition.

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The pre- and postnatal findings are presented in an infant with Russell-Silver syndrome confirmed by methylation analysis of the H19 imprinting centre in the newborn period. The 18 week fetal morphology scan showed asymmetric intrauterine growth restriction with long bone measurements on the 5th percentile for age compared with cranial measurements on the 50th percentile for gestation. The umbilical artery Doppler profile was mildly abnormal. A chromosomal abnormality was excluded. The male infant was delivered at 38 weeks gestation with weight and length significantly restricted compared with a head circumference on the 75th percentile for gestation. He had retrognathia, clinodactyly of the fifth fingers, and proportional limb shortening. Methylation analysis of the H19 imprinting centre showed a reduced methylation ratio in keeping with Russell-Silver syndrome. The typical Russell-Silver phenotype was readily apparent at 3 months of age. Russell-Silver syndrome should be considered with these findings on the antenatal scan. The diagnosis may be confirmed through molecular studies after birth. Further research is required to establish normal methylation ratiosof the 11p15.5 critical region in the second trimester. It will then be possible to test whether the diagnosis of Russell-Silver syndrome can be established at the time of the fetal morphology scan.

P0479. Severe skeletal malformations detected by prenatal ultrasound: estimation of recurrence risk

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Objective: To evaluate estimation of recurrence risk following sonographic detection of severe skeletal anomalies resulting in pregnancy termination.
Methods: We performed a retrospective chart review for a 17 year period at our tertiary referral centre. Twenty-five pregnancy terminations were done at a gestational age of 17-23.9 weeks after sonographic detection of skeletal malformations. Two medical geneticists and one genetic counsellor reviewed all charts independently.
Results: All three investigators agreed upon which investigation: 1. constituted the best basis for diagnosis at the earliest point in time and 2. provided the best basis for estimation of recurrence risk. All diagnostic investigations are shown in Table 1.
Recurrence risk categories were: <1% (n=9), ≤5% (n=9), ≤25% (n=3), ≤50% (n=2). Inter-observer discrepancy in recurrence risk estimation occurred in 2 of 25 cases (one category apart).
Conclusions: Recurrence risk estimates for skeletal malformations detected by prenatal ultrasound examination were in reasonable agreement among the 3 investigators. In 16 of 25 cases a more definitive diagnosis could be reached with the help of additional studies.

Table 1: Investigation(s) giving the most precise diagnosis

Diagnosis	Ultra-sound (n=25)	Autopsy (n=24)	X-ray (n=25)	Autopsy and X-ray (n=24)	History (n=25) Karyotyping (n=10), DNA-analysis (n=1)
Osteogenesis imperfecta (n=9)	6			3	
Thanotophoric dysplasia (n=4)	1		1	2	
Sacrococcygeal teratoma (n=2)	2				
Other (n=10)*		4	1	2	3
Total	9	4	2	7	3

* Multiple congenital anomalies (n=2), trisomy 18 (n=1), osteochondrodysplasia (n=1), rhizomelia (n=1), Kniest dysplasia (n=1), Larsen's syndrome (n=1), ectrodactyly (n=1), cerebrocostomandibular syndrome (n=1) and spinal muscular atrophy (n=1).

P0480. Prenatal diagnosis of spinal muscular atrophy I (Werding-Hoffman disease)

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Spinal muscular atrophy I (SMA I) is the second most common lethal autosomal recessive disorder of childhood, affecting 1 in 6000 - 10000 births, with carrier frequency of 1 in 40-60. It primarily affects the anterior horn cells of the spinal cord and motor cranial nerve nuclei, leading to progressive paralysis with atrophy. The symptoms of the disease start before the age of six months and death generally occurring within the first two years. The gene for SMA has been mapped to chromosome 5q11.2 -q13.3. The molecular analysis of SMN gene in Macedonian patients showed that SMA is associated with high frequency of deletions in exon 7 and 8 of the SMN gene (91.6%)

Prenatal diagnosis is most frequently requested by families with SMA I. In this paper we present the direct DNA analysis of SMN gene performed in eighth families with SMA I. DNA obtained from chronic villi or amniocytes were analyzed for deletions in the SMA gene. The results showed that six fetuses were normal and two fetuses were homozygote for a deletion of exons 7 and 8. After genetic counseling, parents of the six normal fetuses decided to continue the pregnancy. The results were confirmed after birth.

Direct DNA deletion analysis of the SMN gene in affected families represents a highly reliable and fast method for prenatal diagnosis of SMA.

P0481. Differential Detection of α or β-Globin Gene Deletion by Rapid Triplex PCR

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Exact determination being carrier of α-thal or β-thal at the molecular level is crucial for counseling and prenatal diagnosis.

Ten milliliters blood sample was taken from 10 normal and 50 individuals referred to us as being possible carrier of α- or β-thal. DNA extraction by standard protocols, salting out, phenol-chloroform, and magnetic beads were tested. Primers for α-globins were taken from literature and primers for β-globin were designed. Triplex PCR was optimized testing different conditions. Crucial parameters were found to be DNA extraction method, dNTP concentration, and presence of DMSO and glycerol. After electrophoresis quantification of the bands were done by Photocapt software. PCR products with intact (no deletion) gene showed band densities different from persons having deletion in globin genes. Several repeated tests with samples known to have deletion in β- or α-globin genes proved our quantitative method being accurate and useful.

This method reduces the number of specific tests required to identify the type of mutations in globin genes. Non-deletion mutation studies can be facilitated by following these results. In usual multiplex PCR methods used to identify alpha deletion, the reverse primer which is to amplify normal α2 is located in the same site of PA;16-bp del , which results in misinterpretation that the person is homo for α2, though the reality is single deletion plus PA;16bp deletion. In comparison with the other multiplex PCR methods, ours amplify short fragments which are easy to amplify and the band sizes are good enough to be separated on the gel.

P0482. Molecular Analysis of Individuals With Low MCV, MCH, and Normal Hemoglobin A2

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Objective: We tested 30 Iranian individuals with low MCV, MCH and normal HbA2 who had been referred to us by different Primary Health Care centers involved in National Premarital Screening for thalassemia. The aim of the present study was to determine presence or absence of mutations in the alpha or beta globin genes in these Individuals.

Background: Thalassemias are inherited disorders of hemoglobin production characterized by absence or reduction in globin chain synthesis leading to an imbalance of the globin chains.

Material & Methods: DNA were extracted from blood samples and used to perform gap-PCR for α- thalassemia deletions (i.e: $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{Med}$) and sequencing to determine possible mutation in β globin gene.

Results: 11 of these were heterozygote for 3.7 kb deletion and one was compound heterozygote for 3.7kb and 4.2 kb deletions .One showed a SNP at IVS-II-nt711 A◊T in β -globin gene and the other one a hemoglobin variant at Cd: 127A◊G. We can conclude that among these 30 individuals none had mutation in their β-globin gene to warrant prenatal diagnosis after marriage.

Discussion: Molecular analysis of β and α globin gene in 30 individuals suspicious of being β-thal carriers demonstrated that all of them were normal for β-globin mutation and therefore no PND was needed. Although the sample size was not Large enough it can be concluded that individuals with low MCV (mean: 75 ± 4.2), low MCH (24.3 ±3.6) with normal HbA2 (2.51± 0.6) may not be carrier of β-thal.

P0483. Rapid Testing of Embryo, CVS And Amniotic Fluid Samples For Beta Thalassemia Mutations By Real Time PCR And Melting Curve Analysis

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Beta thalassemia is one the most common genetic disorder. Rapid, cost effective and reliable tests for prenatal, carrier and preimplantation genetic diagnosis is of great importance for effective fight against this public health problem. Real Time PCR technology is widespread, sensitive and has low running cost. We have developped protocols and studied 11 CVS, 25 amniotic fluid and 126 blastomers for beta thalassemia mutations: IVS I:110, IVS II:745, Codon 15 , IVS I:6, CD39, CD8/9, CD44, IVS I:5, IVS II:1 and IVS I:1. As low as 1 single villus from CVS; 200 microliter amniotic fluid or single blastomers were sufficient for analysis. The samples were tested in parallel by DNA sequencing. Turnaround time for prenatal samples was 90 min. An initial amplification was required before Real Time PCR set up and therefore the turnaround time was 3 hours for blastomers. Real time PCR by hybridization probes followed by melting curve analysis is a fast, reliable and economic method for prenatal and preimplantation genetic diagnosis of beta thalassemia mutations.

P0484. Results of 102 Prenatal Diagnosis Performed at Pasteur Institute of Iran

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Objective: The aim of this study is giving information to high-risk carriers, establishing a genetic counseling program and prevention of birth of Thalassemic children through carrier testing and prenatal diagnosis.

Background: Thalassemia is an inherited disorder of hemoglobin production characterized by a reduction in globin chain synthesis

leading to an imbalance of the globin chains. The most clinically sever form of B-thalassemia is called Thalassemia major.
Material and Methods: Prenatal diagnosis was performed for 102 couples carrier for β-thalasemia referred primary health care (PHC) centers between 2002- 2005. CVS was obtained at 10-12 weeks of gestation by a specialist.
After characterizing parental mutations by ARMS and performing linkage analysis by polymorphic markers (RFLP), Chorionic villus samples were collected and tested by above methods in duplicates.
Results: Prenatal diagnosis revealed 102 fetuses with 21 (%20.5) affected 54 (%54.9) heterozygotes and 27 (%26.4) healthy fetuses. The most common mutation observed in affected fetuses was IVSII-I(G"A) with frequency %42.8, Fr8-9 (-AA) %23.8, IVSI-5 (G"C) %14.2, I110 (G"A) %9.5, Codon 44 (-C) %4.7, IVSI-25del %4.7, respectively.
Discussion: All of the target population had requested for PND and were willing to accept prenatal diagnosis as a means for control of thalassemia. A nationwide thalassemia control program is in progress and our work is a part of that program.We have to admit that the number of PD performed is not large but the primary role of our center is to act as National Reference Center for the whole country when they seek our help.

P0485. Detection of toxoplasma gondii in amniotic fluid using fluorescent-PCR method

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Background: Intrauterine toxoplasma infection can cause serious congenital malformation. As active fetal infection occurs only in a few percent of pregnancies showing seroconverison during pregnancy, the detection of the infective agent from the amniotic fluid is important to diagnose affected fetuses and determines the further management.
Methods: We tested 64 amniotic fluid samples for the presence of Toxoplasma. gondii using fluorescent-PCR and DNA fragment analysis. DNA was isolated from the amniotic fluid samples by using silica adsorption method. We determined the sensitivity of the method too.
Results: The fluorescent-PCR and DNA fragment analysis has the detection limit of 10-100 parasites. We detected 5 toxoplasma positive samples from the studied 64 amniotic fluids.
Conclusions: Fluorescent-PCR and DNA fragment analysis is a reliable method for the detection of congenital toxoplasmosis, and can be used in diagnosis of acute toxoplasma infection during the pregnancy.

P0486. Global up-regulation of genes on the trisomic chromosome in the developing human

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Human autosomal trisomies remain a common cause of early pregnancy loss, neonatal death and multiple congenital anomalies. Among all the autosomal trisomies, only trisomies of the chromosomes 21 (Down syndrome), 18 (Edwards syndrome) and 13 (Pätau syndrome) are compatible with postnatal survival. Autosomal trisomies are characterized by mental retardation, neurological changes, intrauterine growth retardation, congenital heart defects, and characteristic facial and skeletal features though with considerable variability in the severity and pattern of associated malformations. The variability in each case cannot be predicted by the karyotype alone. There are currently two major hypotheses to explain the phenotypic variability. It is unclear whether the entire transcriptome is disrupted, or whether there is a more restricted increase in the expression of genes assigned to the trisomic chromosome. Both hypotheses are not necessarily mutually exclusive.
Using Affymetrix microarray technology, we examined gene expression in amnion and chorion villus cells of pregnancies with trisomy 21, 18 and 13, and observed chromosome-wide up-regulation in transcription levels for genes assigned to the trisomic chromosomes.

P0487. Prenatal diagnosis in amniocytes of a mixoploid karyotype involving trisomy 3 and trisomy 18

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In chorionic villi discrepancies between the prenatal and fetal karyotype are a well-known phenomenon, occuring in 1-2 % of all pregnancies. The karyotype seen in amniocytes, though, usually well represents the fetus proper.
We present a case with discrepancies between amniocytes and fetal cells as well as different findings with karyotyping of cultured fetal cells and FISH on uncultured cells of the same tissue.
A 22-year-old woman (G1P0) was referred in the 22nd week of pregnancy because of ultrasound abnormalities which were suspect of a chromosomal aberration.
Interphase FISH on uncultured amniocytes with probes specific for the chromosomes 13, 18 and 21 (LSI-13, L1.84 and LSI-21) showed three signals for chromosome 18 in 15 out of 34 nuclei.
The pregnancy was terminated at 23+ weeks. The fetus with a birth weight of 335g showed the typical facial features of the trisomy 18 syndrome. Cultured amniocytes showed a double trisomy. In one cell line a male chromosomal pattern with an extra chromosome 3 was seen and a second cell line showed a trisomy 18.
A discrepancy was seen between the results of cultured cells from ear cartilage (trisomy 18 in all metaphases) and the FISH-results on uncultured cells of this tissue. An extra signal for chromosome 3 only was seen in 52 nuclei, for chromosome 18 only in 21 nuclei and 17 cells showed a normal signal pattern.
A number of issues will be raised regarding the reporting and interpretation of this case.

P0488. A case of true mosaicism for trisomy 22 during first trimester prenatal diagnosis

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Introduction: Trisomy 22 mosaicism is characterized by growth and developmental delay, and a variety of malformations, the severity of which correspond to the percentage of the affected cells with the extra chromosome. We present a case of a fetus with mos 47,XY,+22[3]/46,XY[30] detected during the 1st trimester.
Case Report: A CVS was performed at 13th week to a 30 years-old healthy primigravida woman after the detection of increased levels of β-hCG (6.5 MoM) in the combined screening test. Nuchal translucency was normal (1.0 mm). The results from QF-PCR were normal for 13, 18 and 21 trisomies. Trisomy 22 was detected in 50 cells using conventional GTG banding (47,XY,+22). At 16 weeks' gestation the sonogram revealed intrauterine growth restriction and echogenous bowel, and an amniocentesis was performed. In two different cultures 3/30 colonies with trisomy 22 were detected using the in situ culture method. The pregnancy was terminated after genetic counseling. Post mortem examination showed dysmature and small placenta, histologic signs of hypoperfusion, fetal hypoxia, asymmetrical growth restriction, and increased head circumference. Micropenis and simple (nonscrotal) hypospadias were the only malformations seen. Typical abnormalities of trisomy 22, such as optic coloboma and preauricular pits were absent.
Discussion: Autopsy findings were consistent with the low percentage (5%) of trisomic cells in the fetus, taking into consideration the known phenotypic variability of trisomy 22. It is remarkable that the invasive prenatal diagnosis was undertaken after the detection of extremely high levels of β-hCG at 12 weeks' gestation, before the presence of any sonographic findings.

P0489. Is the role of ultrasound in prenatal diagnosis of aneuploidy crucial? - four cases

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cine, Charles University in Prague, 2 nd Faculty of Medicine, Prague, Czech Republic.

The role of ultrasound imaging techniques in the detection of aneuploidy risk estimation has notably improved over the past years. The ultrasound revealed not quite typical features for certain chromosomopathies.

We present four cases of chromosomal aneuploidy syndromes diagnosed on the basis of ultrasound investigation .

First case was presented by giant cystic hygroma, hydrops universalis , anhydramnion and ascites at the 18 th week of gestation. The cytogenetic investigation revealed karyotype: 47, XY,+21.At the second case large frontal encephalocele was detected by the ultrasound at the 13 th week of gestation, the cytogenetic analysis confirmed trisomy 18. At the third case(also trisomy 18- detected postnatally), congenital heart defect (TOF) and bilateral aplasia of the radius was discovered by the ultrasound at the 20 th week of gestation. The forth case showed on the ultrasound (19 th week of pregnancy) large omphalocele, sandal gap, genital defect and on the fetal echocardiography hypoplastic left heart. Cytogenetic analysis approved the karyotype: 47,XY,+13. The parents of three mentioned cases decided to terminate the pregnancy. At the third presented case parents refused amniocentesis and decided to continue the pregnancy. The proband was born at the 34 th week of gestation, her karyotype was: 47,XX,+18.The upper limbs were severely malformated and congenital heart defect was confirmed. The girl died 7 days after birth.

Imaging techniques have been critical in the diagnostics of aneuploidies, they can provide clinically useful information for the genetic counselling guidiance.

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P0490. Prenatal diagnosis of hereditary vitamin D dependent rickets type I

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Vitamin D dependent rickets type I (VDDR-I) is an autosomal recessive disorder caused by mutations in the 25-Hydroxyvitamin D3, 1α-hydroxylase gene. Clinically it is characterized by hypotonia, weakness and growth failure from early infancy. The affected child was a homozygous for the mutation Pro112Leu (CCC->CTC) in the 1α-hydroxylase gene. Here we present a prenatal diagnosis for the second pregnancy in this family.

Material and methods. Genomic DNA from the fetus was isolated from chorionic villus obtained at 10th week of pregnancy. DNA from the affected child and parents was isolated from the peripheral white blood cells. Part of the 1α-hydroxylase gene was PCR amplified using specific primers. Second PCR was performed using nested specific primers. PCR products were purified and sequenced using Big Dye Terminator v1.1 Cycle Sequencing kit, analyzed on ABI Prism 310 automated sequencer. The chorion DNA purity was analyzed by AmpF1 STR identification kit, on ABI Prism 310.

Results. The sequencing analyses of fetal DNA showed that the fetus is a heterozygous for the mutation Pro112 Leu (CCC->CTC) in the 1α-hydroxylase gene, or only a carrier for the VDDR I. We also performed sequencing analyses of the affected child that showed presence of the mutation on both chromosomes. The STR analyses excluded a possible contamination of the fetal with maternal DNA, and confirmed the paternity, as well.

Conclusion. Although the necessity of the prenatal diagnosis is controversial in this setting, our experience suggests that it is technically possible and it might help for earlier treatment if necessary.

P0491. Prenatal diagnosis of ventriculomegaly: Experience in the prenatal center Chvrpr

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Ventriculomegaly is a nervous system malformation characterized by the dilatation of cerebral lateral ventricles, with an incidence of 0.3 to 1.5 in 1000 births.

The ventriculomegaly may be associated with other central nervous system (CNS) malformations, including spina bifida, agenesis of the corpus callosum. In addition to congenital infections, chromosomal abnormalities are possible causes, and the prognosis depends on the primary aetiology.

From July 1998 to December 2005, all cases of ventriculomegaly at our institution were reviewed. Ventriculomegaly was assumed to be present when atrial width was equal or greater than 10mm.

A total of 18 singleton pregnancies with sonographically determined fetal ventriculomegaly at 20-28 week's gestation were identified. The age of the pregnant women ranges from 21 to 32 years.

Fifteen cases were isolated ventriculomegaly and 3 were concomitant with other CNS malformations, 14 were unilateral and 4 showed bilateral enlargement of lateral ventricle.

The incidence of chromosomal abnormalities was 5.5% and this was in an unilateral isolated ventriculomegaly.

According to our data, a careful ultrasound evaluation and a chromosomal analysis should be offered to patients with ventriculomegaly.

P0492. Familial dicentric Y;22 translocation with no phenotypic effect

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In the most common form of Y/autosome translocation, the heterochromatic portion of Yq is translocated onto the short arm of an acrocentric chromosome, and it is observed in phenotypically normal individuals with normal fertility. Very rarely, the same translocation can result in an unbalanced karyotype with 45 chromosomes. In these cases, the fusion of the Y chromosome and the acrocentric produces a dicentric chromosome. We describe a new case with a familial dicentric Y;22 translocation. An amniocentesis was performed on the second pregnancy of a 28-year-old woman because her husband was carrier of 45,X,dic(Y;22)(q12;p11.2) karyotype. This translocation had been identified by chance during chromosome analysis on a previous pregnancy referred for positive maternal serum screening. Cultured amniocytes showed 45,X,dic(Y;22)(q12;p11.2)pat.ish dic(Y;22)(SRY+, DYZ1+,D14Z1/D22Z1+) karyotype. The dicentric chromosome included the Y heterochromatic region. In all the metaphases examined (n=118) the Y chromosome centromere was inactivated and the chromosome 22 centromere constricted. Ultrasound examination showed a male fetus with normal phenotypical appearance and the pregnancy is going on. This translocation seems to be of no phenotypic or reproductive effect in this family, since the healthy father has transmitted it to his both sons. However, it represents an alternative sex-determining mechanism, which will depend on the segregation of the normal chromosome 22 and the dicentric as well as the transmission of the X chromosome, with a theoretical risk of sex aneuploidy in offspring.

P0493. 18 Years Struggle to Introduce the PND as a Practical and Reliable tool for Prevention of Genetics Disease and Congenital abnormalities. Report of the result of 5270 PND tests

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Progress of hygiene and life style has resulted in a remarkable decrease of mortality and morbidity caused by environmental factors and have led to the pronounced influence on hereditary disorders.

New strategies and approaches were necessitated to deal with the new order. Prenatal diagnosis has become an instrumental preventive technique in the management and control of Genetic disease and congenital malformations.

After the efforts of Iranian geneticists and in spite of major limitations caused by the imposed war; the first amniocentesis for chromosomal aberration in Iran was performed in 1987 (20 year delay), followed by CVS in 1988; first molecular analysis for hemoglobinopathy was first done in 1990 and enzyme analysis for metabolic disorders in 1991. Major other obstacles remained more, namely:

legal approval of therapeutic abortion, obtained in 1997, the ethical and moral issues regarding abortion, overcome after clerical approval

in the same year, and insurance coverage for PND, obtained a couple of years later.
Removing legal, ethical and financial barriers along with increased public awareness in regards to the hereditary disorders have been extremely inductive in increasing the demand for PND and establishment of several PND centers over the country.
We are reporting the results of 5860 PND tests that have been performed at our center for various indications as follow:
Chromosomal Aberration: 4298(Amniocentesis: 4074, CVS: 203, Blood Cord: 21), Hemoglobinopathies (α & β Thalassemia):1255, Myopathies (DMD: 57, SMA:84):141, Metabolic disorders:127, Triple Nucleotide Repeats:24, Skin Lesion:9, Miscellaneous:6.

Po04. Cancer genetics

P0494. Cytogenetic study in acute lymphoblastic leukemia - preliminary results

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Cytogenetics plays an essential role in diagnosis, prognosis and treatment outcome prediction in lymphoid malignancies.
We report the results of a cytogenetic study including 23 cases with acute lymphoblastic leukemia (ALL) at onset. Karyotype analysis has been performed in 65% of the total cases.
There were no microscopically visible chromosomal rearrangements in 33% of the cases, while 67% of them exhibited chromosomal abnormalities:
-numerical changes in 33% of cases (hypodiploidy -20%-; hyperdyploidy -13%-),
-structural cytogenetic anomalies in 20% of cases,
-both numerical and structural aberration in 13% of cases.
The most frequent structural rearrangement was t(9;22)(q34;q11) (13%). Translocation t(8;14)(q24;q32) have been identified in one ALL type 3 case, associated with: trisomy 9, inversion 15, chromosome Y loss and deletions 3q21, 5p12, 9p11.
The relatively high incidence of chromosomal aberration identified in our patients group (67%), in concordance with literature data, highlights once more the value of chromosomal studies in ALL management.
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P0495. Treatment-related AML characterized by t(11;20)(p15;q11) and del(9)(q22)

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With the advent of oncologic therapeutic regimens, cancer is being treated with greater effectiveness, allowing patients to live longer. However, for some patients, oncologic treatment has come with a price, namely, therapy-induced malignancies.
Chromosomal aberrations are considered causal events in the process of leukemic transformation. They are frequently associated with therapy-related myelodysplastic syndromes and acute myelogenous leukemia and are thought to result from exposure to cytotoxic drugs.
We report on a 53-year-old woman, who developed treatment-related secondary acute meyloid leukemia (AML), two years after being treated for diffuse large B-cell lymphoma (DLBCL) with the CHOP-R protocol (Cyclophosphamide**,** **H**ydroxydoxorubicin, **O**ncovin, Prednison+ Rituximab). The leukemia was quite resistant to chemotherapy and was characterized by a unique, previously unreported combination of two cytogenetic abnormalities, namely, t(11;20)(p15;q11.2) and del(9q) .
The precise mechanism by which alkylating agents induce leukemias is unclear, but it is possibly related to chromosomal damage causing mutations or translocations of genes implicated in cellular growth and differentiation. Specific DNA alterations may lead to a survival advantage of a pluripotent cell that eventually encourages expansion of the malignant clone. Translocation t(11;20)(p15;q11.2) is a rare but recurrent translocation that has been reported in patients with MDS , AML , polycythemia vera and in a subset of nontherapy-related acute myelocytic leukemia. Del (9q) is a known recurrent cytogenetic abnormality in acute myeloid leukemia (AML). The co-occurrence of these two, recurrent cytogenetic abnormalities is reported here for the first time. We suggest this combination may confer a poor outcome.

P0496. Diagnostic value of fluorescence in-situ hybridization (FISH) for detection 11q23 rearrangements in childhood acute myeloid leukemia

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Childhood acute myeloid leukemia (AML) is a heterogeneous disease the prognosis of which is depends on spectrum of genetic abnormalities detected in tumor cells. The aberrations of MLL gene located on 11q23 are associated with unfavorable prognosis of AML. Effective revealing of 11q23 rearrangements is important for treatment correction and outcome prediction.
The aim of the study was the evaluation of diagnostic significance of fluorescence in-situ hybridization for detection of 11q23 rearrangements in childhood acute myeloid leukemia.
Total 95 children with de novo AML treated in Research Center for Paediatric Oncology and Haematology (Minsk, Belarus) from 1999 to 2004 were enrolled in the study. Standard cytogenetic analysis using G-banding was carried out in all cases. FISH analysis was performed using two kinds of probes: LSI MLL dual-colour DNA probe (11q23) (VYSIS, USA); WCP for human chromosomes 10 and 11 (Oncor Appligene, France).
The 11q23 rearrangements were detected in 17 patients (18%) with AML. FISH confirmed the finding of standard cytogenetics in 8 cases (47%), revealed cryptic translocations t(10;11)(p12;q23) in two patients with no evidence of 11q23 aberration by G-banding, defined the ambiguous chromosomal abnormalities in one case with t(9;11)(p22;q23),one case with t(6;11)(q27;q23) and in one case with deletion of 11q23 region. Moreover, FISH with WCP probes for chromosomes 10 and 11 disclosed the complex mechanisms of translocation t(10;11)(p12;q23) in four children with AML.
So, FISH method is essential tool for revealing of cryptic 11q23 rearrangements and allows to increase the efficiency of detection prognostically valuable chromosomal abnormalities.

P0497. Interaction of isoform products of the RIL gene with α-actinin and its potential role in actin cytoskeleton rearrangement

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Adapter proteins participate in regulation of cell growth, adhesion, and malignant transformation. The gene for reversion-induced LIM domain adapter protein RIL expresses several alternative transcripts. The major transcript encodes a PDZ-LIM protein implicated in actin stress fiber dynamics. Minor transcripts lack certain exons resulting in either LIM domain substitution by a 23aa peptide due to a frame shift or loss of PDZ domain. We overexpressed Flag-tagged alternative proteins and a truncation mutant lacking LIM domain. RIL variants with alternative C-terminus could not be detected by immunoblotting due to rapid turnover while there was no difference in mRNA and translation levels. Fusion of the C-terminal 33aa alternative peptide to EGFP reduced fluorescence intensity and protein half-life some 10-fold. Thus, the alternative C-terminus confers instability to the whole RIL protein. It was previously shown that RIL interacts with α-actinin via its PDZ domain. We performed co-immunoprecipitation assays with Flag-tagged RIL variants. As expected, RILdeltaPDZ did not bind to, while full-length RIL only weakly coimmunoprecipitated with α-actinin. Nevertheless, RIL variant with the alternative C-terminus and RIL truncation mutant exhibited dramatic increase in interaction with α-actinin. We speculate that loss or modification of LIM leads to greater affinity of PDZ domain to α-actinin. Moreover, in case of RIL with alternative C-terminus two additional bands of 27 and 55kD recognized by anti-α-actinin antibody were detected. These data suggest a role for RIL alternative splice variant in regulation of α-actinin degradation. By this mechanism RIL might regulate cytoskeleton rearrangement in cancer.

P0498. Identification of new mutations of the APC gene and analysis of the MYH gene in FAP and Colorectal cancer patients

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The Familial Adenomatous polyposis (FAP) in an autosomal inherited syndrome characterized by numerous colorectal polyps at a very young age which normally confers a high risk for colorectal cancer development. APC is also mutated in >85% of sporadic colorectal tumors. Germline mutations in the APC gene (located in chromosome 5q21) result in FAP. Inactivation of the APC protein by increased mutation rate and chromosomal instability determines both tumor formation and progression. Recently, It has been found that germ-line mutations in the MYH gene (located in chromosome 1p34) are responsible for a FAP-like condition and that biallelic germ-line mutations could increase the risk for colorectal cancer development.

In the present study we have identified nine novel mutations in the APC gene in 36 FAP and 26 sporadic colorectal cancer patients: Five polymorphisms (silent transitions in exons 11, 13 and 15 of the gene), one missense mutation in exon 15, two insertions in exon 15 and two deletions in exons 7 and 15. Most of these mutations are located within the ß-catenin binding and downregulation domains which are invloved in adhesion and cellular migration and favour tumor development and progression. Additionally, we found a FAP patient who carries a biallelic mutation in the MYH gene with no APC mutation.

P0499. PAX8 expression in human bladder cancer

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The transcription factor PAX8 is an important determinant for thyroid development and its role has been also demonstrated in the ontogenesis of kidney. Aim of this study was to investigate expression of PAX8 in normal bladder and in non invasive urothelial tumours. Several cases of normal urothelial mucosa and non invasive papillary urothelial carcinomas of low and high grade were investigated by immunohistochemistry. PAX8 mRNA expression was evaluated by RT-PCR in a different set of normal bladder mucosa and tumours. In addition, by RT-PCR, PAX8 expression was evaluated in bladder from human embryos and in several continuous cell lines derived from bladder tumours (5637, RT-112, TCC-SUP, HT 1376). In immunohistochemical studies, PAX8 was expressed in 26 out of 28 non invasive urothelial tumours, but never in normal adult bladders. In RT-PCR studies, PAX8 was expressed in 13 out of 13 bladder tumours but not in 6 normal bladder mucosa. Differently than in adults, PAX8 was expressed in 2 cases of bladder mucosa from 16 week-old embryos. PAX8 was expressed in all cell lines from bladder tumours. Both in bladder tumours and cell lines PAX8 expression was highly heterogeneous in terms of splicing isoforms. Treatment of cell lines with Sodium butyrate (NaB) induced several changes of the splicing isoforms. These data indicate that PAX8 is expressed in most non invasive bladder tumours but never in normal bladder mucosa. Moreover only subsets of molecular events that determine the PAX8 mRNA splicing heterogeneity are sensitive to NaB treatment.

P0500. *MDM2* T309G polymorhism is associated with bladder cancer

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Recently a functional T to G polymorphism at the nucleotide 309 in the promoter region of MDM2 gene has been identified. This polymorphism has an impact on the expression of the *MDM2* gene, which is a key negative regulator of the tumor suppressor molecule p53. We hypothesized that this gene polymorphism might be a critical predisposition factor for bladder cancer, as MDM2 molecule is an important player in bladder cancer pathogenesis evidenced by its over-expression in 30% of urothelial carcinomas. Bladder cancer is a major cause of morbidity and mortality. In the Turkish population it is the third most common cancer in men and eighth in women. We studied the effect of T309G polymorphism of the *MDM2* gene on bladder cancer susceptibility in a case control study of 75 bladder cancer patients and 103 controls of the Turkish population. Genomic DNA was isolated from 200 µl blood by standard phenol-chloroform extraction. *MDM2* T309G polymorphism was determined by polymerase chain reaction and restriction digestion. The G/G genotype exhibits an increased risk of 2.68 (95% CI, 1.34-5.40) for bladder cancer compared with the combination of low-risk genotypes T/T and T/G at this locus. These results show an association between *MDM2* T309G polymorphism and bladder cancer in our study group. To the best of our knowledge, this is the first study reporting that *MDM2* T309G polymorphism could be a potential genetic susceptibility factor for bladder cancer.

P0501. Molecular genetic alterations in human bladder carcinoma.

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Bladder cancer is one of the most common malignacies in developed countries. Although it is potentially curable in the majority of cases, the prognosis for patients with advanced disease at presentation is poor. Molecular genetic tests increse the sensitivity of tumor detection and diagnostics. We investigated 25 pT1 transitional cell carcinomas DNA sample pairs (15 samples with initial cancer, 10 with relapses) of bladder cancer (tumor and normal tissue) for loss of heterozygosity (LOH) at p53 region (chromosome 17p), FGFR3 (7th, 10th exons) mutations and the abnormal methylation of p16 and CDH1 genes.

In a group of initial cancer samples FGFR3 mutation (s249c, 7th exon) was found in 33% (5/15) cases, hypermethylation of p16 in 20% (3/15) and of CDH1 27% (4/15), p53 loss of heterozygosity was identified in 6% (1/15) cases. In the second group the same typical FGFR3 mutation was detected in 20% (2/10) cases, p16 and CDH1 were methylated in 40% (4/10), 50% (5/10) accordigly; p53 LOH was detected in 30% (3/10) cases. Molecular genetic alterations were more characteristic for the cases with often relapses, except the FGFR3 mutations, which might be associated with more favourable prognosis.

P0502. BRCA1 and BRCA2 germline mutations in male breast cancer patients in the Czech population

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Breast cancer is a rare disease in men. The proportion of male breast cancers attributable to BRCA mutations has not yet been determined with accuracy. The estimated mutation carrier frequencies varied from 4% to 40%.

We have determined the contribution of germline mutations in the BRCA1 and BRCA2 genes to the pathogenesis of male breast cancer in Czech republic by screening a series of 14 unselected male breast cancer patients. The average age at diagnosis was 61 years.

The entire coding regions and intronic splice sites of both BRCA1 and BRCA2 were screened using heteroduplex analysis and protein truncation test, followed by direct sequencing of abnormalities. Four of the 14 male breast cancer cases (29%) were observed to carry pathogenic mutations. One in BRCA1 (frameshift mutation c.5385dupC) and 3 in BRCA2 (frameshift mutations c.8138_8142delCCTTT, c.9631delC and splice site mutation c.7235G>A). The average age at diagnosis of mutation carriers was 69,5 years and three of the mutation carriers reported a positive family history of breast or prostate cancer. We also identified rare variants of unknown significance in four patients - 2 in BRCA1 gene and 2 in BRCA2 gene.

Our results provide evidence for a strong genetic component of male breast cancer in Czech republic and support the recommendation that male breast cancer patients should be offered genetic counseling and

testing.
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P0503. Ocurrence of ten unclassified variants of BRCAs genes in spanish control population

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A great number of germ-line mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 have been identified in breast cancer families. Besides the disease-causing germ-line mutations a great deal of variants of unknown pathological significance have been found in both genes. The functional consequences of this variants remains unclear. Our aim was to determine the presence or absence of ten of these unclassified missense mutations in control population from Spain. One of them appeared in the BRCA1 gene: G1201S, and the rest in the BRCA2 gene: H150R, D244N, P357S, S384F, W395G, D935N, K1057R, R2034C, I2840V. Samples were obtained from fifty healthy unrelated individuals of the general population who never had developed any kind of cancer. DNA from blood samples was isolated using standard procedures. Mutational screening was performed by CSGE, restriction endonucleases and automated sequencing to confirm the alterations. Only one of the 10 variants (R2034C) was detected in healthy population and should be considered a polymorphism. More studies are needed to confirm and enlarge these results.

P0504. Detection of two novel genomic rearrangements in *BRCA1* gene in Greek breast/ovarian cancer families using quantitative multiplex PCR of short fluorescent fragments (QMPSF)

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The identification of genomic rearrangements in breast/ovarian cancer families has widened the mutational spectrum of the *BRCA1* gene, thus increasing the number of informative patients who can benefit from molecular screening. Up to date a total of more than 30 different *BRCA1* genomic rearrangements with mapped breakpoints, in all exons of the gene with the exception of the last exon 24, have been reported. The proportion of *BRCA1* mutations due to genomic rearrangements in different populations vary from 8 to ~30%, probably due to both ethnic diversity and the technical approach employed. In order to estimate the contribution of *BRCA1* genomic rearrangements to hereditary breast/ovarian cancer (HBOC) predisposition in Greek families we screened 95 families negative for point mutations or small insertions/deletions in *BRCA1* and *BRCA2* genes using Quantitative Multiplex PCR of Short Fluorescent Fragments. We identified two large deletions of 4.2 and 4.4 kb in exons 20 and 24 respectively. This is the first report of an exon 24 deletion. On the basis of previous and present analyses, rearrangements represent 5% (2/40) of all mutations in our set of *BRCA1* Greek families.

P0505. A case of constitutional mosaicism for a *BRCA1* mutation.

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A 31 year old woman visited our family cancer clinic because she suffered from breast cancer. There was no family history of breast- or ovarian cancer on either the maternal or paternal side. We screened *BRCA1* and *BRCA2* by DGGE in DNA isolated from peripheral blood leukocytes. A novel pathogenic mutation c.784C>T (p.Gln262X) in exon 11 of *BRCA1* was detected. The carrier status of the parents was assessed and revealed that the mutation was of maternal origin. The mother, aged 67, had never suffered from breast- or ovarian cancer. Strikingly, DGGE analysis of mother's DNA from peripheral blood leukocytes showed an imbalance between normal and mutant alleles, with a minority of mutant alleles. A similar ratio of normal and mutant alleles was found by direct sequencing. Subsequently we investigated DNA isolated from a mouthwash and from cultured skin fibroblasts. The ratio between normal and mutant alleles from the mouthwash was comparable to the ratio observed in peripheral blood leukocytes. However, in the skin fibroblasts hardly any mutant alleles were detected. In conclusion, the mother of our index patient is mosaic for the c.784C>T mutation, and hence this mutation must have originated *de novo* in the mother. To our knowledge this is the first report of a mosaicism for a *BRCA1* mutation. We will look for further proof for the mutation being *de novo* and a possible germcell mosaicism by assessing the inheritance of *BRCA1* alleles in siblings and children of the mosaic woman respectively.

P0506. BRCA1 intragenic rearrangements in patients with hereditary breast and ovarian cancer syndrome in the Czech Republic

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Germline mutations in highly penetrant cancer susceptibility genes BRCA1 and BRCA2 cause genetic predisposition to breast and ovarian cancer. Absence of pathogenic mutations in BRCA1 and BRCA2 genes in some high-risk families can be due to the presence of intragenic rearrangements as deletions, duplications, alternatively insertions of one or more exons in these genes. Methods used for mutation screening of BRCA1/2 genes based on PCR of genomic DNA enables the detection of point mutations, small insertions or deletions, but difficulties arise in detecting of large intragenic rearrangements.
There are no available data relating to the type and frequency of genomic rearrangements in BRCA1 gene in patients with hereditary breast and ovarian cancer syndrome in the Czech Republic. Multiplex Ligation-Dependent Probe Amplification (MLPA) has been applied for examination of BRCA1 rearrangements. Using Salsa P002 BRCA1 MLPA kit (M.R.C. Holland) DNA from 152 high-risk patients with no previously found BRCA1/2 mutation was investigated. Six different deletions were identified: exon 1A/1B and 2 deletion, partial deletion of exon 11 and exon 12, exon 18 and 19 deletion, exon 20 deletion, exon 21 and 22 deletion and deletion of exon 5 to 14. These deletions represent almost 6% of all BRCA1/2 mutation-negative cases and nearly 8% of all detected BRCA1/2 mutations. The precise determination of the change on DNA and mRNA level is ongoing.
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P0507. Haplotype and linkage disequilibrium analysis of BRCA1 and BRCA2 genes: identification of a hemizygous region of BRCA2 in a German high risk breast cancer familiy

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As a member of the "German Consortium for Hereditary Breast and Ovarian Cancer" we were interested in understanding the linkage disequilibrium (LD) pattern and the haplotypic structure of the BRCA1 and BRCA2 genes among German women who were previously tested by direct sequencing of all exons and exon-flanking intronic regions of both genes. We therefore performed a haplotype-based study of BRCA1 and BRCA2 based on 22 single nucleotide polymorphisms (SNPs) spanning 80.78 kb of the BRCA1 gene and 25 SNPs spanning 83.01kb of the BRCA2 gene. Out of five different BRCA1 haplotypes with a frequency of > 5%, two common BRCA1 haplotypes accounted for two thirds of all chromosomes in our collective. In contrast, analysis of BRCA2 haplotypes revealed a higher haplotypic diversity where the most abundant haplotype reached a frequency of 17%. We

also assessed the pattern and the extent of LD between the SNPs in BRCA1 and BRCA2 and compared the results to data from the International HapMap Project. While most methods agreed on a single coherent region of elevated LD that spanned most of BRCA1, BRCA2 showed low levels of LD in general and only a single area of strong LD. Furthermore, an unusual BRCA2 haplotype was detected in a German high-risk breast cancer familiy. This haplotype is characterized by the loss of a high-LD area in BRCA2 and represents a hemizygous region that results from a disease-causing intragenic deletion. In conclusion, SNP-based haplotype analysis can be an effective approach for identifying intragenic deletions leading to hemizygosity.

P0508. BRCA2 variant associated with modification of ovarian cancer occurrence among Russian carriers of BRCA1 mutations.

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Russian breast/ovarian cancer families have high prevalence of one mutation - 5382insC - in BRCA1 spectrum (75% in analyzed sample of 60 carriers). Therefore, breast or ovarian cancer risk associated with BRCA1 mutations can be modified by other than mutations position genetic factors.

We studied some variants of BRCA2 as a candidate for a risk modifier gene under BRCA1 mutations. Single nucleotide polymorphisms N372H and located in the 5'-untranslated region variant 203G/A were investigated. The frequency of BRCA2 203GA heterozygous variant for group of women with ovarian cancer was less than for group of women with breast cancer, both carrying a BRCA1 mutations (OR=0.28; P=0.026). There were no significant difference between these groups with N372H variant. In control sample (n=109) the frequency of 203A rare allele was 0.25 that corresponds to earlier estimation. The association of BRCA2 203GA variant with ovarian cancer was significant on one-side Fisher exact test (OR=0.39; P=0.043) and no association was observed with breast cancer (P=0.27). It is intriguing that 203AA homozygous variant was more frequent in ovarian cancer group in comparison with control group (OR=6.9; P=0.023). The frequency in breast cancer group was not differ from control (OR=1.14; P=0.9). Futher investigations are necessary for conclusion.

P0509. Analysis of Cytogenetic and Biochemical parameters in Breast Cancer females with Metastasis in Coimbatore City, Tamil Nadu

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Breast cancer is the most common malignancy next to ovarian cancer among females. The etiology of this carcinoma is attributed to several factors. In the present study total of 95 confirmed cases of Carcinoma of Breast with metastasis were selected from the local hospitals and equal number of healthy non-pregnant females were used as controls. A statistically significant increase was noted in the level of ALP (Alkaline Phosphatases) and GGT (Gamma Glutamines Transferase), whereas non-significant results were observed with GSH (reduced Glutathione) and LDH (Lactate Dehydrogenase) when compared to that of controls. Karyotypic analysis among 95 female cancer patients only 37 of them (38.95%) displayed Chromosomal aberrations such as 46, XX t(12p$^-$; 15q+); 46, XX t(5p$^-$; 13q+), 46 XX, t(1q$^-$;10p+) and deletions in the short and long arm of Chromosomes 3, 6, 13, 17. among controls only two of the subjects displayed deletions of short / long arms of chromosome 1 and 22. the data were discussed pertaining to the recent literature available.

P0510. Mutation screening of BRCA1/2 and CHEK2 in 480 unselected breast cancer patients from Russia

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Mutations in BRCA1/2 genes are associated with greatly increased risk for the breast cancer (BC) development. The estimated lifetime risk of developing BC is from 60 to 85% for BRCA1 and from 60 to 80% for BRCA2 mutations. The CHEK2 1100delC variant, by contrast, is associated with much lower (2-4 fold) risk of BC. In Russia, the contribution of BRCA1/2 and CHEK2 mutations in unselected BC population is largely unknown because only few and limited surveys have been carried out up to now. The aim of the study was to characterise the BRCA1/2 and CHEK2 mutations frequencies in unselected BC patients from Russia using hybridization with oligonucleotide biochips. The following mutations were selected for the analysis: 185delAG, 300T>G, 4153delA, 4158A>G, 5382insC in BRCA1gene, 695incT, 6174delT in BRCA2 gene and 1100delC in CHEK2 gene. 480 woman diagnosed with BC and unselected for family history of the disease have been studied. The following mutations were identified: in BRCA1 gene, 185delAG in 4 patients, 300T>G in one patient, 4153delA in 2 patient, 5382insC in 16; in BRCA2 gene a 695insT mutation was detected in one patient, 6174delT in 1; in gene CHEK2 a 1100delC mutation was detected in 8 patients. Altogether, a total of 23 mutations (4,8%) in BRCA1 gene, 2 mutations (0,4%) in BRCA2 gene were detected. The freguency of CHEK2 1100delC variant was 1,7%. The most prevailing mutation was 5382insC (69,5% of all BRCA1 mutations). These results will be of significance for both diagnostic testing and epidemiological studies.

P0511. Breast cancer and HumARA CAG polymorphism

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The role of HumARA polymorphism in the breast cancer predisposition was analyzed in several studies with conflicting results. A decreased risk has been observed with smaller repeat lengths, in particular among post menopausal women. Other studies, including young women and different cut-off points, found no associations. Recently a reduced risk with shorter CAG repeats only among women with a positive family history of breast cancer was demonstrated. Nevertheless, comparing CAG repeat lengths in cancer tissues and in surrounding normal tissues, instability in tumor associated alleles with contractions and expansions of CAG repeat was reported.

To provide additional data we have tested 50 incident cases of breast cancer from Caucasian women analyzing the tumor and the near healthy tissues, both typed for forensic genetic profiles.

For the relatively small sample size, that doesn't permit to distinguish true association from false positive results, no conclusion about correlation between CAG length and breast cancer cases compared to control population, was possible. MSI occurred in cancer tissues influencing the identification of the samples and in MSI-H cancers HumARA was highly instable. Besides the data agree with a recent report in which this marker is considered not suitable for identification purposes.

P0512. Male Breast Cancer - the challenge to develop special prevention strategies

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We discuss a pedigree example in regard to the importance to develop special strategies of prevention in BRCA positive males in families with hereditary breast and/or ovarian cancer. The diagnosis of male breast cancer is rare and if a mutation is found in affected males it is usually a BRCA2 mutation. If such a mutation is revealed in breast cancer risk families the question of prevention strategies is not only a question concerning the female family members, but also a very important question for the still healthy and BRCA2 mutation positiv analyzed male family members. These males are at a higher risk than a male of the average population to get breast cancer and immediately the question concerning prevention strategies arises. In the literature there are recommandations to perform prevention as well as necessary treatment similar to strategies concerning females but there are no specialized health centers for males with longterm experiences. While

numerous specialized “Breast Cancer Centers” for females are rising up, it might be important to discuss and develop prevention strategies especially for males.

P0513. High-throughput SNP typing of low penetrance candidate genes of sporadic breast cancer

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Linkage analysis has failed to identify the genetic causes of familiar breast cancer. A polygenic model has been proposed in order to explain the genetic susceptibility to breast cancer. According to this model, common population variants would be responsible for a modest to low effects of risk to develop sporadic cancer.

Here we have carried out a high-throughput SNP genotyping project in order to shed some light on the complex genetic aetiology of non-familiar breast cancer. For this, SNPlexTM (Applied Biosystems) highthrouput genotyping platform, which allows the study of up to 48 SNPs simultaneously, was used for the study of 676 SNPs belonging to 89 genes putatively related to sporadic breast cancer, in a total of 480 female cases of breast cancer and 480 female controls.

Genes have been selected mainly because their implications in several candidate cell pathways for breast cancer, such as genes related to BRCA1-dependent Ub-ligase activity or genes related with the role of *BRCA1*, *BRCA2* and *ATR* in cancer susceptibility. SNP were selected following a direct/indirect approach criterion that allows capturing an important amount of the variability of these candidate genes. An independent set of neutral SNPs have been genotyped in order to control for population stratification that could lead to spurious positive association results. We have identified a total of 28 SNPs with *P*-values below 0.05 (under a Chi-square test) that would deserve further investigation in independent studies. We noted however that these associations do not find support when applying appropriate corrections for multiple tests.

P0514. Analysis of the functional roles of c-Myc-dependent regulating ribosomal DNA genes transcription

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The c-Myc oncoprotein regulates transcription of genes that are associated with cell growth, proliferation and apoptosis. Proteasome inhibition leads to c-Myc accumulation within nucleioli, indicating that c-Myc might have a nuclolar function. Previously, we showed that proteins c-Myc and Max interact in nucleoli and are associated with ribosomal DNA, that c-Myc is required for activating rDNA transcription in response to mitogenic signals, and that c-Myc-mediated rDNA transcription is RNA Polymerase II independent. Furthermore, we investigate the functional roles of c-Myc in regulating rDNA transcription. The results suggest that c-Myc not only recruits chromatin modifying complexes (TRRAP, Tip60, GCN5) changing silent chromatin structure but also recruits RNA polymerase I machinery cofactors (RNA polymerase I, SL-1, UBF) binding to promoter of rDNA. Intriguingly, the binding pattern of c-Myc to rDNA leads to the assumption that c-Myc should be involved in gene looping regulation of rDNA transcription.

P0515. Low frequency of gross rearrangements in the two known BRCA genes in breast/ovarian cancer families from Germany

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The vast majority of alterations identified in the BRCA1 or BRCA2 gene are either point mutations or small insertions/deletions. Large genomic rearrangements could frequently be determined in some populations. However, preliminary studies on a limited number of German families suggest that such mutations are rare in our population. We therefore screened 571 affected females from different risk groups for rearrangements in BRCA1 and 200 females at high risk for BRCA2 deletions.

In agreement with previous studies, we identified low frequencies for BRCA1 large deletions. Eight different gross aberrations were found in 15 out of 571 index patients (2,6%). However, we saw regional and risk-dependent differences. While 11 mutations were identified in 279 families living in North Rhine-Westphalia, only three deletions were found in 272 affected females from Southern Germany (chi2-test, p=0.034). Furthermore, most of the aberrations were detected in high-risk families with three or more cases of breast cancer, including two premenopausal ones and in families with breast and ovarian cancer (12/321 = 3,7%). A novel deletion of exon 8 was found in an ovarian cancer familiy (1/15 = 6.7%). So far, no large rearrangements have been found in the BRCA2 gene in 200 high-risk families. This agrees with another study from Germany who also failed to detect gross aberations in 150 high risk families. Thus, our study strongly indicates that screening for deletions in Germany is only indicated in the BRCA1 gene in high risk families.

P0516. Expression of a new cancer/testis gene ,TSGA10, in Iranian patients with gastrointestinal tumors

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Cancer-testis (CT) genes are a group of genes expressed in male germinal cells and certain tumor types. There is a numerous researchs on tumor antigens, which could be used to direct the potent cytolytic capacities of the human immune system against cancer. TSGA10 is expressed in normal testis, but neither in a variety of other normal tissues.This gene could be classified as a member of the cancer-testis (CT) gene family. We studied TSGA10 overexpression in gastrointestinal (GI) tumors by semiquantitative RT-PCR. Out of 31 patients, 18/31(58.1%) showed TSGA10 transcripts. Males showed 12/22(54.5%) and females showed 6/9(66.7%) TSGA10 expresstion. GI epithelial tumors with 18/30(60%) TSGA10 positive and one mesenchymal tumor with no TSGA10 expression were seen. TSGA10 expression were seen in 10/20(50%) of GI adenocarcinomas and 7/9(77.8%) of SCCs. It also were seen in 11/15(73.3%) of samples with lymph node involvement and 6/9(40%) of samples without lymph node involvement. Our results suggestes that TSGA10 can be a sutable tumor marker for gastrointestinal cancer diagnosis and may be potentially useful for cancer immunotheraphy.

P0517. Microarray analysis identifies up-regulation of CD36 in human PBMC treated with endocannabinoids

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This is a study of the effect of anandamide and arvanil on human peripheral blood mononuclear cells (PBMC). We analyzed the regulation of gene expression under immunosuppressive condition. During PBMC proliferation many genes are induced or repressed while others are constitutively expressed. We used microarray technology to identify a regulatory pattern associated with cell proliferation in the presence of anandamide and arvanil. PBMC stimulated by CD3-CD28 showed a pattern of up-regulated and down-regulated genes after treatment with arvanil and anandamide. We selected and further analyzed several genes by real time PCR and protein expression analyses in order to validate the results observed by microarrays. The genes were chosen by their function in the regulation of cell proliferation. In particular CD36 showed an increased expression when treated by both arvanil and anandamide. Our results suggest a possible role of CD36 in anandamide and arvanil anti-inflammatory pattern.

P0518. Quantitative determination of human telomerase reverse transcriptase (hTERT) mRNA expression in premalignant cervical lesions and correlation with HPV load

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Human telomerase reverse transcriptase (hTERT) mRNA expression and HPV 16 viral load were quantified using real-time PCR and were correlated with cytologic findings and the presence of HPV infection in cervical specimens. hTERT mRNA expression was evaluated in 15/73 (20.5%) atypical squamous epithelial cells of undetermined significance (ASCUS), in 62/156 (39.7%) low grade squamous intraepithelial lesions (LGSILs), in 49/51 (96%), high grade squamous intraepithelial lesions (HGSILs) and in 9/45 (20%) normal samples, while viral load was quantified in 52/58 (89.6%) samples infected with HPV-16. The mean levels of hTERT mRNA expression were 0.11 in normal tissue, 0.23 in ASCUS, 0.75 in LGSIL and 2.5 in HGSILs. A significant increase in quantitative hTERT mRNA expression was observed with increasing degree of cervical dysplasia. The HPV-16 viral load was significantly higher in samples with HGSIL than LGSIL ($p<0.001$). A significant correlation was observed between viral load and quantitative hTERT mRNA expression values ($r=0.65$, $p<0.05$). Quantitative hTERT mRNA assessment showed 96% sensitivity and 100% negative predictive value (NPV), while specificity and positive predictive value (PPV) were 72% and 36.2% respectively. Based on the observed moderate specificity and high sensitivity and NPV, it can be suggested that quantitative hTERT may be used as an adjunctive marker in the management of women with high grade cervical dysplasias.

P0519. Monitoring of chimerism after allogeneic bone marrow transplantation using STR-PCR techniques

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Bone marrow transplantation (BMT) is a medical procedure that involves transplantation of hematopoietic stem cells (HSC) for patients with inborn or obtained life-threatening diseases of the blood or bone marrow.

The main goal of post-transplantation monitoring in hematopoietic stem cell transplantation (HSCT) is to predict negative events, such as disease relapse, graft rejection and graft-versus-host disease, in order to intervene with appropriate therapy. In this context, by quantifying the relative amounts of donor and recipient cells present in the peripheral blood sample, it can be determined if engraftment has taken place at all or if full or mixed chimerism exists.

In this work we present our results after one year of following chimerism in our group of patients who underwent the allogenic BMT by related donors. Our aim is to establish the protocol for monitoring chimerism in our pediatric patients, to determine the optimal interval between two analyses and to compare the predictivness of mixed chimerism for relapse in different hematological diseases.

For this purpose we decided to use standard human identfication tests, based on multiplex PCR analyses of short tandem repeats (STRs), as they are highly informative, sensitive (1-5%), fast and therefore represent an optimal methodological approach for engraftment analysis.

P0520. 1p36 LOH and expression study of apoptotic genes in a selected group of chordomas

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Chordoma is a rare embriogenetic neoplasm arising from notochordal remnants and characterized by physaliferous cells. Common genetic lesions in chordoma are: 1p36, 3p, 12p losses; 1q, 7q, 9q gains. An LOH study on 1p36 region revealed a high incidence of 1p36 losses among sporadic chordomas (85%). Until now, no molecular characteristics have proved to be useful in establishing prognosis.

We performed an 1p36 LOH study on 16 chordomas for which strict clinical follow-up was available and determined the expression of apoptotic genes mapping in the region.

We tested 33 microsatellites spanning from D1S243 to D1S478. 12 chordomas (75%) showed LOH, 3 of which displaying an LOH region involving all the tested markers. 4 patients showed segmental LOH, probably because of the presence of clones with different LOH extents in the same surgical sample. The 4 remaining patients (25%) didn't display LOH and showed the most indolent clinical course.

We also determined the expression profile of TNFRSF1B; TNFRSF8; TNFRSF9; TNFRSF14; CASP9; TP73; DFFA and DFFB versus that of nucleus pulposus. No chordoma showed an expression profile matching that of nucleus pulposus. 12 showed TNFRSF1B expression, while seven expressed TNFRSF8; seven TNFRSF9 and nine TNFRSF14. In 14 we detected CASP9 expression. DFFB was expressed by 11 chordomas. Only one patient lacked DFFA expression. TP73 was never expressed, with the exception of two patients.A correlation between collected data and clinical follow-up is under study, in order to highlight elements that might be useful in assessing a correct prognosis in chordoma patients.

P0521. Monitoring of minimal residual disease in chronic myeloid leukemia

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Chronic myeloid leukemia (CML) is a clonal stem cell disease characterized by the presence of bcr-abl fusion gene resulting from reciprocal chromosomal translocation t(9;22)(q34;q11) . bcr-abl gene was proved to play the principal role in CML pathogenesis .

The treatment of CML has been revolutionized by Imatinib. More than 80 % of newly diagnosed patients with chronic myeloid leukemia in chronic phase will achieve a complete cytogenetic response (CCR) with the standard dose of 400 mg imatinib daily.

Minimal residual disease monitoring is important for assessment of disease status in patients who obtain a complete cytogenetic remission to detect early signs of relapse. Quantitative RT-PCR assays enable to monitor the kinetics of residual bcr-abl transcripts over time.

Serial peripheral bcr-abl m-RNA levels were monitored by quantitative real time PCR using the Taq man probe system for 32 CML patients in chronic phase for a median of 14 months after they achieved complete cytogenetic remission.

The patients could be divided into three groups : in 16 Patients transcript levels continued to decline during the period of observation , in 11 patients the transcript levels reached a plateau and in 5 patients transcript numbers rises . rising levels of bcr-abl are strongly predictive of cytogenetic and hematologic relapse .

We conclude that the pattern of residual disease after achieving complete cytogenetic remission on imatinib is variable The goal of early detection of residual disease is to allow timely therapeutic intervention before overt relapse of therapy resistant disease occurs.

P0522. Complex chromosomal abnormalities in chronic myeloid leukemia with promyelocytic transformation - case report

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Although t(9;22)(q34;q11) is the sole cytogenetic anomaly in a majority of patients with chronic myeloid leukemia (CML) in chronic phase, 60-80% of cases show additional abnormalities in blastic phase. Promyelocytic transformation in CML is a rare event, and t(15;17) is usually identified.

We present the case of a 61 year-old female with promyelocytic transformation of CML, and complex numerical and structural cytogenetic abnormalities.

The hematologic distinctive feature of chronic phase was persistent thrombocytosis, resistent to therapy. Blastic crises occurred after 3 years of evolution.

Bone marrow chromosomal studies identified Philadelphia translocation as unique anomaly, in blastic phase. Glivec therapy has been started, but primary resistance was observed.

Cytogenetic reevaluation, at 12 months of Glivec therapy, revealed additional abnormalities: two Philadelphia chromosomes, trisomy 9, monosomy 15 and derivative chromosome 3. The whole missing chromosome 15 is translocated on the distal region of 3q and generates an extra long derivative chromosome 3.
In our case, no t(15;17) was observed, instead chromosome 15 is involved in another rearrangement. FISH will be used in order to elucidate the events that generated the complex genomic abnormalities involving chromosomes 3 and 15, in promyelocytic transformation.
The mechanisms of blastic transformation in CML are still poorly understood. As more cases are investigated, novel chromosomal anomalies might reveal new insights into the cytogenetic-molecular mechanisms that led to acute transformation.
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P0523. Rare recurrent translocation t(1;3)(p36;q21) in chronic myeloid leukemia - case report

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The reciprocal translocation t(1;3)(p36;q21) have been reported in various myeloid malignancies characterized by megakaryocytic hyperplasia, dysplasia and a poor prognosis.
In this paper we present the case of a 41year-old female patient with chronic myeloid leukemia (CML), harbouring both t(9;22)(q34;q11) and t(1;3)(p36;q21).
Bone marrow chromosomal studies at diagnosis, in blastic phase, showed t(9;22)(q34;q11). The patient reentered chronic phase after chemotherapy, with thrombocytosis as distinct feature, and subsequently received interferon and cytarabine therapy.
The cytogenetic reevaluation at 12 months after diagnosis, revealed the acquisition of an additional rearrangement: t(1;3)(p36;q21). At least four different clones were observed: 46,XX/ 46,XX,t(9;22)(q34;q11)/ 46,XX,t(1;3)(p36;q21),t(9;22)(q34;q11)/ 46,XX,-1,+der(1)t(1;3)(p36;q21),t(1;3)(p36;q21),t(9;22)(q34;q11).
Further characterization of the breakpoints cluster regions at 1p36 and 3q21 should provide important insights into the molecular genetic mechanisms involved in the genesis of t(1;3)-positive malignancies, and may contribute to the understanding of the clinical features associated with this rearrangement.
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P0524. Aberrant circadian gene expression in hepatocellular carcinoma

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Circadian rhythm plays an important role in the regulation of digestive system. The human circadian rhythm is controlled by at least nine circadian genes. The aims of this study are to understand the aberrant expression of the circadian genes between hepatocellular carcinoma (HCC) tissues and non-tumor tissues, and to explore the possible mechanism(s) contributing to the difference. We analyzed differential expression of the 9 circadian genes in 46 HCC and paired non-cancerous tissues by real-time quantitative RT-PCR and immunohistochemical detection. We also tested the possible regulatory mechanism(s) by direct sequencing and methylation PCR analysis. Our results showed that decreased expression levels of *PER1, PER2, PER3, CRY2* and *TIM* in HCCs were observed. Down-expression of these genes was not caused by genetic mutations, and 34.8% of the down-regulated cases were caused by promoter methylation. The down expression of circadian genes may result in disturbance of cell cycle, but there is no relationship between the level of down expression and the clinical status of patients. In conclusion, down-regulation of circadian genes results in disturbance of circadian rhythm in HCC which may disrupt the control of the central pacemaker and benefit selective survival of cancerous cells and promote carcinogenesis. It is possible that the disturbance of circadian rhythm influence the cell cycle as well as provide survival benefit for HCC. The differential expression of circadian genes between non-cancerous and cancerous cells may provide a molecular basis for chronotherapy of HCC.

P0525. Application of the hot spot BRAF mutation (V600E) in the HNPCC diagnostic strategy

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Colorectal cancer (CRC) is one of the leading cancer diseases in the Western world as well as in Bulgaria. In the development of the disease, genetic and environmental factors are involved. Hereditary Nonpolyposis Colorectal Cancer (HNPCC) accounts for 5-8% of all colorectal cancers and has been linked to mutations in DNA mismatch repair (MMR) genes, resulting in genetic alterations as microsatellite instability (MSI). Approximately 12-17% of the sporadic cases also show MSI but MMR mutations are unfrequently found. BRAF gene encodes a serine/threonine kinase and plays an important role in the mitogen-activated protein kinase signaling pathway. Recently, an oncogenic V600E hotspot mutation within BRAF, has been found to be associated with sporadic colorectal cancer with MSI, but not with HNPCC.
The aim of this study was to analyze the frequency of this hotspot mutation in our group of patients and the applicability of this analysis in the HNPCC diagnostics. A total of 140 patients with CRC (39 sporadic and 101 familial cases) with known MSI status have been studied. The V600E mutation has been detected by SSCP analysis and direct sequencing.
The BRAF-V600E hotspot mutation has been found in 6 of the MSI and in 2 of the stable tumors. A strong correlation has been found between the mutation and the sporadic origin of the tumors (p<0,002), supposing that screening for MMR mutations in cases positive for BRAF mutation can be avoided. We propose the involvement of this simple, low cost and applicable analysis in the HNPCC detection strategy.

P0526. Low Frequency of *AXIN2* Mutations and High Frequency of *MUTYH* Mutations in Patients With Multiple Polyposis

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Background & Aims: Familial adenomatous polyposis has been linked to germline mutations in the *APC* tumor suppressor gene. However, a number of patients with familial adenomatous polyposis (with classical or attenuated phenotype) have no *APC* mutation. Recently, germline mutations in the *Wnt* pathway component gene *AXIN2* have been associated with tooth agenesis-colorectal cancer syndrome. Moreover, biallelic mutations in the base excision repair gene *MUTYH* have been associated with polyposis and early-onset colorectal cancer. The aim of this study was to further assess the contribution of *AXIN2* and *MUTYH* to hereditary colorectal cancer susceptibility. **Methods:** *AXIN2* and *MUTYH* genes were screened entirely for germline mutations by PCR and direct sequencing in 39 unrelated patients with multiple adenomas or colorectal cancer without evidence of *APC* mutation nor mismatch repair defect. **Results:** Two novel *AXIN2* variants were detected in one patient with multiple adenomas, but no clearly pathogenic mutation. In contrast, nine different *MUTYH* mutations were detected in eight patients, including six novel mutations. Biallelic *MUTYH* mutations were found only in patients with multiple adenomatous polyposis (7 of 22 (32%)). Interestingly, four *MUTYH* mutation carriers had a family history consistent with dominant inheritance. Moreover, one patient with biallelic *MUTYH* mutations presented with multiple adenomas and severe tooth agenesis. **Conclusions:** Germline mutations are rare in *AXIN2* but frequent in *MUTYH* in patients with multiple adenomas. Moreover, our data suggest that genetic testing of *MUTYH* may be

of interest in patients with family history apparently compatible with recessive as well as dominant inheritance.

P0527. Cytogenetic contribution in diagnosis of leukemia

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The valuable contribution of cytogenetics in diagnosis, classification and risk group ascertainment at the onset of leukemia it is well recognized.

In this paper we present a review of cytogenetic investigation performed on 288 leukemia cases : 260 myeloproliferative disorders (chronic myeloid leukemia = CML, acute myeloid leukemia = AML, other chronic myeloproliferative disorders = CMPD and myelodysplastic syndrome = MDS) and 28 acute lymphoblastic leukemias (ALL). Bone marrow cells were processed directly or after short-term cultivation. Classical techniques revealed besides recurrent rearrangements, new, unknown abnormalities. The cytogenetic results contributed at: diagnosis confirmation in CML Ph-positive cases; diagnosis reconsideration in Ph-negative cases of myeloproliferative disorder; association of Ph chromosome with essential thrombocytemia in 4 cases and identification of new cytogenetic abnormalities in 5 patients. A variant Philadelphia translocation was identified in one case, with the following karyotype: t (3;9;22)(3q25::9q34;9q34::22q11;22q11::3q25). FISH results with dual fusion, dual color probes were consistent with the results obtained by conventional cytogenetics. Other 5 cases had additional or unusual abnormalities like: -t(1 ;1) in CML at debut; - t(13 ;21) in AML-M2 ; -t(6 ;14) in a case with myelodysplastic/myeloproliferative disorder at first presentation; -t(3 ;5) in ET at diagnosis; -t(7 ;9) in ALL at the onset. The global percentage of unusual, additional and variant abnormalities was 21%. It must be underlined the higher incidence of chromosomal abnormalities in acute leukemias (85%) compared with chronic myeloproliferative disorder (48,6%). The mechanism and prognostic impact of variant and additional abnormalities have to be elucidated.

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P0528. Analysis of homozygous or combined deletion 13q4.3 in patients with B-cell chronic lymphocitic leukemia

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B-cell chronic lymphocitic leukemia (B-CLL) is the most frequent leukemia in the Western world. Deletion of chromosome 13q14.3 is the most frequent genetic aberration in B-cell chronic lymphocitic, founded in approximately 50% of patients with B-CLL and suggesting the presence of tumour suppressor gene(s) whose loss or inactivation may contribute to the pathogenesis of B-CLL. Loss of this region has also been observed in other human malignancies. The 13q14.3 deletion can be found in the onset of B-CLL, in contrast to other chromosomal aberrations. The deletion can be heterozygous, homozygous or combined. Deletion 13q4.3, as sole anomaly, has a good prognosis in clinical evolution, but not data are available about possible clinical repercussion of homozygous or combined form.

Routinely FISH analysis, with specific probes for chromosomes 11 (LSI ATM), 12 (CEP12), 13 (LSI D13S319/13q34) and 17 (LSI p53), were performed 270 samples from B-CLL patients. A 53% of samples showed the del(13)(q14.3) in hemizygous, homozygous or combined form.

The implication of homozygous or combined deletion in hematologic features and clinical follow-up of these B-CLL patients will be discussed.

P0529. Investigation of DNA Ligase I Gene Polymorphism in Patients with Laryngeal Carcinoma by PCR-RFLP Technique

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Laryngeal squamous cell carcinoma (LSCC) is the one of the most common cancer type on the worldwide. Altough in the arising of this disease environmental factors like as smoking, work stiuation, life styles are effective, many cellular mechnisms especially DNA replication, recombination and repair systems may be contribute to disease development. DNA ligase I is an essential enzyme that required for DNA replication, recombination and repair processes. A single base exchange (A→C) in exon 6 of DNA ligase I gene were reported. So we hypothesised that polymorhism of gene which encodes this enzyme can impress the cancer development. In this study, we evaluated that total 234 genomic DNA material for PCR-RFLP analysis; obtained from 94 patients with laryngeal carcinoma and 140 healthy controls. DNA ligase genotype frequencies were 17%, 47,9%, 35,1% in patients and 22,1%, 39,3%, 38,6% in controls for AA, AC and CC, respectivly. We did not find any significance differencies between LSCC and DNA ligase I gene polymorphism in patients and control group (χ^2=1,886, P =0,389). However a significant difference were found between smoking habits (χ^2= 6,256 and P= 0,044), nodular metastasis (χ^2= 9,544 and P=0,049) and DNA ligase I gene genotype. In the literature, there is no study about LSCC and DNA ligase polymorphisms, so our study is the first research this subject.

P0530. Nuclear Localization of the Human Mismatch Repair Proteins, MLH1, PMS2, and MLH3

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DNA mismatch repair (MMR) mechanism eliminates DNA polymerase errors from the newly synthesized strand during replication and recombination. Consistent with the role of MMR proteins in the maintenance of genomic stability, loss of MMR function predisposes to cancer. MMR process is proposed to consist of three steps, mismatch recognition and assembly of the repairosome, degradation of the error-containing strand, and repair synthesis. In man, one key factor in the assembly of the repairosome is suggested to be MutLα, a heterodimer of MLH1 and PMS2. Another heterodimer of MutL homologues MLH1 and MLH3, MutLγ, was recently suggested to assist in the repair of base-base mismatches and single extrahelical nucleotides. Consistent with the functions, MMR proteins are mainly localized in the nucleus. Two different proteins, MLH1 and a transcription factor p73, have been shown to influence the subcellular distribution of PMS2 and dimerization of MLH1 and PMS2 has been shown to limit nuclear localization of MutLa. Whether nuclear localization of MLH1 is limited or influenced by its dimerization with PMS2 or MLH3 as well as nuclear localization of MLH3/ MutLγ has remained questionable. Here, by using normal human MLH1, PMS2, and MLH3 proteins and mutated MLH1 proteins, which were unable to interact or lacked nuclear localization signal (NLS), we demonstrate that protein interaction is essential to nuclear import of PMS2 but not of MLH1. In the presence of MLH1 and PMS2, MLH3 protein seems to locate in cytoplasm.

P0531. Donor cell-derived acute myeloblastic leukemia after allogeneic peripheral blood hematopoietic stem cell transplantation for juvenile myelomonocytic leukemia

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The recurrence of leukemia following allogeneic hematopoietic stem cell transplantation (HSCT) is one of the major consequences of the treatment failure. It frequently results from outgrowth of residual host tumor cells; however, in a minority of cases, it may arise from cells of donor origin. Donor cell leukemia (DCL) accounted for approximately 5% of relapses demonstrated by cytogenetic studies of 54relapses in sex-mismatched bone marrow transplants.The improvement in cytogenetic and molecular analysis such as short tandem repeat (STR) sequencing have provided more accurate and faster determination of donor engraftment and also differentiation of DCL from host leukemia relapse.

We report here the case of a 5 year-old girl with juvenile myelomonocytic leukemia (JMML) and normal female karyotype who developed acute myeloblastic leukemia (AML) with a karyotype of 46, X, t(X; 7) (p21;

p11.2), der(7)t(3; 7) (q13.3; q22) five months after peripheral blood hematopoietic stem cell transplantation (HSCT) from her HLA-matched sister. We performed the hybridization of short tandem repeat sequence markers to DNA obtained from donor peripheral blood, patient's peripheral blood including leukemic blasts and patient's hair root. This hybridization showed that the leukemic blood DNA matched the donor blood DNA and not the patient's DNA, thus confirming DCL
To our knowledge this is the first case of a donor cell leukemia developing after peripheral blood HSCT forJMML.

P0532. Mutation analysis of the EGFR tyrosine kinase domain in Spanish Head and Neck Cancer patients.

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The epidermal growth factor receptor (EGFR), a tyrosine kinase, regulates a number of essential cellular functions and appears to play a central role in the aetiology and progression of a variety of solid tumours. EGFR is frequently overexpressed in several epithelial tumours including head and neck carcinoma (HNC), thus being a promising therapeutic target. In fact, inhibition of activated protein kinases has emerged as an effective approach to cancer therapy.
Recent studies showed that the mutations in the kinase domain of *EGFR* gene in non-small cell lung cancer (NSCLC) tissues could predict significant clinical responses to tyrosine kinase inhibitors (TKIs).
The different involvement of *EGFR* mutations in several types of cancer and the correlation between mutations and clinical benefits prompted us to examine the presence of mutations in exons 18, 19, 20 and 21 in *EGFR* for HNC Spanish patients.
Genomic DNA was extracted from 31 tumoral tissues of HNC samples and analysed by PCR-SSCP. DNA sequence variations in *EGFR* were found in exons 18, 20 and 21, for 9 out of the 31 of HNC patients (29%). All the variations detected were polymorphisms or were located in non-coding regions. Moreover, they were not involved in the generation of alternative splicing sites as we checked using the FGENESH software.
In our set of HNC patients, mutation in the kinase domain of the *EGFR* gene is a relatively rare event. It would be interesting to investigate the *EGFR* gene amplification and other ways in which the EGFR may be affecting carcinogenesis.

P0533. Expression analysis of embryonic stage specific antigens SSEA-1, SSEA-3, and SSEA-4 in glioblastoma samples: a pilot study

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The 38 samples of glioblastoma tissues were used to investigate the expression of SSEAs. Unfixed samples were stored in a liquid N_2-tank. The immunofluorescence analysis was carried out on tissue pieces taken from frozen samples with a scalpel, fixed to nitrocellulose paper, blocked, and incubated with SSEA specific monoclonal antibodies. Specific reaction with tissue material was revealed by fluorescence microscopy using Alexa conjugated secondary antibodies . The analysis revealed practically all possible SSEA expression patterns in glioblastoma samples except the variant were SSEA-3 is the only expressed antigen (see Table). The most frequent pattern (in 12 of 38 glioblastomas, 31,6%) was co-expression of all SSEAs. The further study should show how much the expression characteristics of SSEAs revealed could be related to the malignisation process of brain cells or used in subtyping of gliomas or explained the pathogenesis. The study is supported by Estonian SF grant nr. 5250 and target grant TARMPO421

Glioblastoma samples: number, (%)	The expression patterns *		
	SSEA-1	SSEA-3	SSEA-4
3	-	-	-
3	+	-	-
6	+	+	-
12	+	+	+
9	+	-	+
3	-	-	+
2	-	+	+
In total: 38	30 (79.0)	20 (52,6)	26 (68,4)
* - negative reaction; + a clear positive reaction with at least in some parts of tissue material.			

P0534. A 7 year survey on familial adenomatous polyposis patients in Switzerland: Identification of novel APC germline mutations and genotype-phenotype correlations

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Familial adenomatous polyposis (FAP), caused by germline mutations in the *adenomatous polyposis coli* (*APC)* gene, shows considerable phenotypic heterogeneity. This variability has been associated with the position of the *APC* mutation: attenuated FAP (AFAP; patients with <100 colorectal polyps) has been related to *APC* mutations at the extreme 5' (codon 1-168), 3' end (codon >1680) and exon 9.
For this study 101 unrelated FAP patients were consecutively screened for germline mutations in *APC*. In 28 (71.8%) out of 39 FAP patients with ≥100 adenomas and 28 (45.2%) out of 62 with <100 adenomas a pathogenic *APC* mutation was identified, with 17 (30.4%) of them representing novel sequence alterations.
The median age at diagnosis of the 56 *APC* mutation carriers was 40.0 years (IQR 18.0) with 50.0% presenting with >100 adenomas. A positive family history was reported in 31 patients implicating 41.5% of the mutations having occurred *de novo*. Extracolonic disease was observed in 19 (33.9%) patients.
Interestingly, 26 (51%) patients carrying an *APC* mutation located within the "classical FAP" region displayed an attenuated phenotype which cannot be explained by earlier age at diagnosis (median 41.0 vs. 36 years, p=0.33). In 5 (8.9%) patients the *APC* mutation was located within the presumed AFAP region (median age: 45.8 years (IQR 6.0)). In contrast to the 2 patients with 3' mutations and, as expected, <100 polyps, all 3 patients carrying a 5' mutation actually displayed hundreds of polyps. Thus, our findings challenge the prevailing view on genotype-phenotype correlations in FAP.

P0535. Subgroups of patients with familial adenomatous polyposis without APC mutations distinguished by genetics and phenotypic characteristics.

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Mutations in MYH can not explain all cases of familial adenomatous polyposis (FAP) without APC mutations. In our sample of Russian patients 55% of FAP were due to APC and 4% due to biallelic MYH mutations. There are some evidence that the patient group without APC mutations is not homogeneous. We divided this group in two subgroups: with not more than one affected relative (subgroup I) and with many affected relatives of a proband (subgroup II). Such subdivision was based on the notion of different FAP risk in the families. Attenuated FAP was observed in 43% of cases in the subgroup I that is significantly higher in comparison with the subgroup II (OR=9.0; P=0.037) and with the APC mutation patient group (OR=10.9; P=0.007). The average age of the disease onset in the subgroup I was 36.2 year that is 1.5 times higher than in the group of patients with APC mutations (t = 2,83; P = 0,007). On the other hand, the difference in average age between patients of subgroup II and patients with APC mutations was not significant.
Further we analyzed four APC polymorphisms in attempt to find specific

gene variants for subgroups. One of the genotype frequency was significantly higher in the subgroup I in comparison with control group (OR=4.9; P=0.015). There was no difference between the subgroup II and control group.

P0536. Fanconi anemia FANCD1/BRCA2 displays genotypic/ phenotypic correlations: a report of two pedigrees and review of the literature.

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Patient 1 had small stature, microcephaly, microphthalmy, cleft palate, VSD, imperforate anus, rectovaginal fistula, absence/hypoplastic thumbs and hypothyroidism. She died at age 3 of a solid tumor in the posterior fossa. Chromosome and complementation studies made the diagnosis of FA type D1. DNA-analysis, reported by Howlett *et al*. *Science* 2002, revealed biallelic mutations in the BRCA2 gene: 7699 insAT and 9900 insA as the cause of type D1 in this kindred (EUFA423). Each parent was carrier. Her deceased paternal grandmother was the only relative with cancer (BRCA2 mutation was absent in the pancreatic tumor).

Patient 2 developed a Wilms' tumor at age 1. She had small stature, microcephaly and café au lait spots. A BRCA2 mutation (886 del GT) had been found in her mother, counseled for familial breast cancer (BC). In her father's family several women had BC, a 9345G>A BRCA2 mutation, reported to have an effect on splicing, was present. Both were present in the child which, together with an increased chromosome breakage, confirmed the diagnosis. Complementation studies are underway.

Twenty two other children have been reported. Distinct clinical features from the 24 patients with FANCD1/BRCA2 are: high incidence of solid tumors in early childhood,10 patients had brain tumors and six a Wilms' tumor, absence of aplastic anemia, early onset acute leukemia, imperforate anus and BC among relatives.

Little correlation between FA complementation groups and clinical features had been reported so far, here we show that genotype/ phenotype associations do exist for this subtype, with potential important therapeutic implications.

P0537. Germline exon- and whole gene deletions in the APC gene and their correlation with phenotype

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During the period 1994-2004 a total of 564 patiënts were submitted for identification of the family specific APC mutation in polyposis patients to our DNA diagnostic laboratory. These patients were screened for germline mutations in the APC gene by Denaturing Gradient Gel Electrophoresis (DGGE), Protein Truncation Test (PTT), sequencing analysis and Multiplex Ligation-dependent Probe Amplification (MLPA).

In 221 patiënts we could identify a pathogenic APC mutation (39%). Most of the mutations in the APC gene were frameshift mutations (n=108, 49%) and nonsense mutations (n=73, 33%), furthermore we found splice mutations (n=20, 9%) and complete or partial gene deletions (n=20, 9%). Also, the Ile1307Lys variant, which is found in 6% of the Ashkenazi Jewish population and confers a relative risk of 1.5-2.0 for colorectal cancer was found in 4 patiënts. One of these appeared to be a carrier of two MUTYH mutations, and one was also carrier of a nonsense APC mutation.

Most of the 20 deletions identified by MLPA were found only once. The recurrent deletions were the whole gene deletions (n=7), exon 9-15 (n=3), and exon 7-13 (n=2). The majority of the APC deletion patients displayed the classical FAP phenotype (100-1000 colorectal adenomas), however, the two families carrying the deletion from exon 7-13 seem to show a more atypical FAP phenotype (less polyps and late onset). Interestingly, only this exon 7-13 deletion is an inframe deletion.

Further investigations are necessary to reveal whether there exists a genotype-phenotype correlation in this ‚deletion carrying' subgroup of patients.

P0538. Analysis of CDH1 and IL1RN variants among gastric cancer families in Russia

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Mutations in CDH1 gene were associated with inherited gastric cancer among patients of several countries. However the mutations origin and spreading are not known. Further investigations of CDH1 gene among patients of different populations can give necessary information.

All CDH1 exons were screened among probands of 9 families with diffuse inherited gastric cancer and 11 with familial gastric cancer by SSCP and CSGE. Revealed variants of DNA fragments were sequenced on both strands. Four rare variants (531+10G>C, 1896C>T, 2076C>T, 2253C>T) and one new variant (1937+23G>A) were found. Frequencies of these rare variant and frequent polymorphism 2076C>T were not differ from earlier described. No deleterious mutations were revealed in CDH1 gene. This result may reflect low frequency of CDH1 mutations among Russian families with inherited gastric cancer.

The association of sporadic gastric cancer risk with some variants of IL1RN gene was established earlier while a role of this gene in familial gastric cancer was not studied. We compared a distribution of IL1RN variants among our sample of familial gastric cancer and control individuals (n=57). No significant association of either IL1RN variant with gastric cancer risk was found.

P0539. Genetic heterogeneity in gliomas

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Gliomas display a wide range of histopathological features and biological behavior, and an inherent tendency to progress to a highly malignant phenotype. Molecular- and cytogenetic studies have delineated that different grades of gliomas correlate with specific genetic alterations. Glioblastomas, the most malignant form of gliomas, may develop de novo (primary glioblastomas) or through progression from low-grade or anaplastic astrocytomas (secondary glioblastomas).

Cell culture analysis may be biased by clonal selection artifacts. Homogenized tissue lacks control over the tissue composition and permits contamination of the tumor specimen with preexisting and reactive nonneoplastic tissue. Moreover, gliomas exhibit a diffuse infiltrating growth pattern into normal brain so that no tumor area contains a uniform cellular composition. Genetic heterogeneity can hardly be detected by conventional methods. In order to evaluate an intratumoral genetic heterogeneity we performed FISH investigations and microdissection analysis in paraffin-embedded glioma tissue Using this method we examined 124 tumor areas from 41 gliomas. Furthermore we correlate the cytogenetic data with the histomorphology of the given tumor areas.

Low-grade astrocytomas most often showed normal karyotypes, by conventional cytogenetic methods. However, we were able to identify numerous alterations in low-grade astrocytomas, especially in areas with a gemistocytic appearance. Primary glioblastomas and secondary glioblastomas showed consistant as well as different genetic findings, which correlate partial with the histomorphological features of the investigated areas. Our results provide clear evidence of inter- and intratumoral genetic heterogeneity in gliomas. In order to develop genetic prognostic criteria, the distinct genetic heterogeneity of these tumors should be considered.

P0540. Glutathione S-transferases null genotype in acute myeloid leukaemia

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Background: The expression of many of the cancer susceptibility enzymes is genetically polymorphic in human population. The glutathione S-transferase (GST) is one of the metabolising enzymes, which plays an important role in the detoxification of mutagens and carcinogens. Different studies have shown an increased frequency of *GST*-null genotypes in several malignancies. **Objective:** To investigate the rate of *GSTT1* and *GSTM1* null genotypes and to determine its importance in prognosis of the disease in AML patients. **Methods:** DNA was extracted from peripheral blood or bone marrow of 180 patients who were in presentation status of AML. A multiplex PCR method was used simultaneously to amplify regions of *GSTM1*, *GSTT1*, and *βετα-globin* genes in genomic DNA. The survival curves were analyzed by the Kaplan-Meier method and compared by the log-rank test (Mantel-Cox) using the SPSS software program. **Results:** Of the total of 180 patients, 23 cases (12.8%) showed null genotypes in both genes, while in 52 patients (28.9%) both genes were wild-types. *GSTM1* null-*GSTT1* wild-type was detected in 91 patients (50.6%) and *GSTM1* wild-type-*GSTT1* null genotype was detected in 14 patients (7.8%). These rates are within the upper limit of the rates detected in the normal European population. There was no significant difference in the overall survival and in disease free survival between different groups. **Conclusion:** These observations suggest that the inherited absence of the *GSTT1* and *GSTM1* carcinogen detoxification pathway may be related to carcinogenesis but it is not an important determinant of prognosis in AML.

P0541. Nonsense associated exon skipping is not sufficient to suppress cancerogenesis in Gorlin syndrome

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Mutations of the transmembrane transporter protein Patched (Ptc), a member of the Hedgehog pathway regulating homeostasis of cell proliferation and differentiation in a variety of tissues, are implicated in the development of autosomal dominant Gorlin syndrome (NBCCS, nevoid basal cell carcinoma syndrome) characterized by multiple skin and endodermally derived cancers as well as congenital abnormalities. Tumor promotion is thought to be associated with reduced functionality of ptc leading to deregulation of downstream targets like sonic hedgehog and Gli pathways. However, transcriptional events leading to the reduced suppression effects of patched have not been studied in detail. We describe a heterozygous germline polymorphism P1315L in a patient suffering from Gorlin syndrome, which contrasts to the bi-allelic, somatic "two-hit" mutations G1019X (sharing the same allele with P1315L) and I1070M in genomic DNA of a basal cell carcinoma (BCC) in this patient. Despite the stop codon present in the BCC, real-time RT-PCR and western blotting showed an over-expression of ptc in tumor cells. This upregulation is mediated by in-frame skipping of the exon 19, harbouring G1019X, thus constituting a failing rescue attempt of the transcriptional machinery to escape the consequences of this premature stop-codon. These molecular alterations underscore the significance of structurally unaltered ptc protein for suppression of cancerogenesis.

P0542. glutathione s-transferases (gstt1 and gstm1) gene deletions: susceptibility and prognostic implications in Breast carcinoma patients

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Glutathione S- transferase (GSTT1 and GSTM1) detoxify a wide range of environmental carcinogens contributing to tumor cell survival by detoxification of products induced by cancer therapy. A study is designed to investigate the susceptibility and prognostic implications of the GSTT1 and GSTM1gene deletions with breast carcinoma in 100 patients compared to 100 controls. The patient group also consisted of ten cases with a positive family history. The mean age at diagnosis in the familial group was 38.0 years as compared to 44.7 years in the sporadic group. The mean age at menarche was 13.5 years in the familial group as against 14.7 years in the sporadic cases.The cancer had metastasized and lymph nodes were affected in 50% of both familial and sporadic cases, but in the sporadic cases, 77.8% of the lymph node positive cases were seen in the pre-menopausal women. Bilateral affection of the disease was seen in only three cases. DNA was screened for BRCA1 gene mutations by CSGE. None of the patients showed BRCA1 gene mutations.GSTT1 and GSTM1 genes were secreened for null mutations. The patients included in the study had primary breast carcinoma and were on chemotherapy. Follow-up was 2 years (range, 3- 24 months).GSTT1 null mutation was associated with risk of early onset of breast cancer. Three patients had a relapse and one patient died from breast carcinoma. The gene deletion of GSTs in relation to early onset of breast carcinoma and clinical response to chemotherapy and recurrence free survival will be discussed.

P0543. Use of FFPE samples on Agilent's oligo aCGH microarrays

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Formalin-fixed, paraffin-embedded tissue samples (FFPE) provide an important source of archival DNA for which extensive clinical data are available. The ability to analyze this material allows retrospective studies of pathogenesis, treatment, and patient outcome. Agilent's oligonucleotide aCGH platform was used to identify regions of chromosomal gain and loss on several cancer FFPE samples. Comparison of matched FFPE and fresh samples from the same patient shows a high degree of correlation for both cell lines and tumor biopsies. Archived and frozen DNA from the same biopsy sample had greater than 90% concordance. To test reproducibility, single normal and hepatocellular carcinoma FFPE samples were processed 4 times. All normal DNA samples showed no aberrations; all hepatocellular carcinoma DNA samples showed the same 2-fold copy number loss at chromosome 9p22. Furthermore, because the amount of patient material available is often limited, and unbiased amplification of the degraded nucleic acids found in most FFPE samples remains a serious challenge, we have developed a protocol that permits the use of as little as 500 nanograms of input genomic DNA in aCGH experiments. The robustness of this system has been tested with FFPE samples from several different tumor types.

P0544. Molecular characterization of splice site mutations in BRCA1 and BRCA2 genes in Czech families with hereditary breast cancer.

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Accurate RNA splicing involves conserved sequence motifs at the intron-exon junctions and the branch site. Splice site mutations often occur at the essentially invariant GT and AT dinucleotides located respectively at the start of an intron (splice donor) or at its end (splice acceptor). Flanking these important signals, however, other conserved sequence elements are present, which, if mutated, can also cause aberrant splicing.

Molecular genetic analysis of BRCA1 and BRCA2 is performed in 500 Czech high-risk breast and/or ovarian cancer families and 150 early-onset cancer cases during the period 1999-2005. In about 30% of our families unequivocal deleterious mutations were identified, including frame-shift, nonsense and missense mutations in the C3HC4 - RING domain of BRCA1. Furthermore, we detected 10 different alterations in conserved splice sites (termed by traditional BIC nomenclature):

BRCA1: c. 421-3C>G; c.4304G>A; c.4794+1G>A; c.5271+2dupT;

BRCA2: c.703G>A; c.704-2A>G; c.7235G>A; c.8983-1G>A; c.9346-2A>G; c.9345+2T>A;

Some of them were reported in the BIC database as "Splice" mutation, but without known effect at the cDNA level. For genetic counseling and clinical management of the patients it is very important to know if changes lead to aberrant splicing. We used splice prediction programs to predict possible changes in efficiency of splice sites and

characterized the transcripts at the cDNA level.
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P0545. Efficiency of the Revised Bethesda Guidelines (2003) for the Detection of Mutations in Mismatch Repair Genes in Austrian HNPCC Patients

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The clinical diagnosis of hereditary non-polyposis colorectal cancer (HNPCC) is based on the Amsterdam II criteria (ACII). The purpose of using the Bethesda guidelines (BG) is to select tumours for microsatellite analysis. Recently the modified Amsterdam criteria (ACmod) and Bethesda guidelines (BGmod) were proposed to simplify definitions. We evaluated the efficiency of the ACmod and BGmod to identify patients with germ-line mutations in MLH1 and MSH2 in 81 unrelated Austrian HNPCC families. Microsatellite (MS) analysis was performed in 55 tumours. The new criteria included more families than the old ones: BGmod, n=81; BG, n=72; ACmod, n=52 and ACII, n=35. The more stringent old criteria tended to show greater positive predictive value for association with a germ-line mutation than the corresponding new criteria: BGmod, 23%; BG, 26%; ACmod, 31% and ACII, 37%. The larger number of patients analysed in the ACmod group resulted in greater sensitivity compared to the ACII. The increased workload for BGmod was not associated with greater sensitivity. Microsatellite instability (MSI) significantly enhanced specificity in all subgroups. We recommend the use of the ACmod criteria to select patients for primary sequence analysis, when microsatellite analysis is not possible. If the BG are used, we suggest that BG be given preference over BGmod, as the former signify a lesser workload.

P0546. Altered Mut L homologue *(MLH1)* mRNA allele expression in hereditary nonpolyposis colon cancer (HNPCC) patients measured using MALDI-ToF MS.

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Germline mutations in the mismatch repair (MMR) genes are known to cause susceptibility to HNPCC, an autosomal dominant disorder that accounts for approx. 5% of all colorectal cancers. About 50% of HNPCC mutations in *MLH1* and the Mut S homologue 2 (*MSH2)* result in the generation of a premature termination codon (PTC). Transcripts containing such mutations trigger nonsense-mediated mRNA decay (NMD) a cellular mechanism that prevents the formation of truncated proteins, which may be detrimental. We have examined *MLH1* transcripts extracted from peripheral blood lymphocytes from HNPCC patients and controls, for altered allelic mRNA expression. All individuals were heterozygous for the exon 8, c.655A>G polymorphism. A primer extension assay was carried out on genomic and cDNA, and products were analysed using matrix assisted laser desorption/ionisation - time of flight mass spectrometry (MALDI-ToF MS). The relative mRNA expression of each allele was quantified and normalised against genomic DNA. We found that there was no change in *MLH1* mRNA allele expression in our controls or in HNPCC patients with missense mutations. However, a 1.99 ± 0.11 fold (mean ± S.E.M.) imbalance in allele mRNA expression was observed in patients with *MLH1* mutations predicted to cause NMD. Interestingly, individuals with a mutation that results in a PTC in exon 2 followed immediately by an ATG codon have a greatly reduced, but still measurable imbalance in *MLH1* mRNA allele expression, 1.345 ± 0.07 fold (mean ± S.E.M.) These results show the potential that this assay has to be used as a pre-screening tool.

P0547. Digenism in a case of early onset colorectal cancer (HNPCC, Lynch syndrome)

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Lynch syndrome or Hereditary NonPolyposis Colorectal Cancer is an hereditary cancer susceptibility caused by alteration in genes involved in postreplicative mismatch repair (MMR), MLH1, MSH2, MSH6. People carrying a mutation in one of these genes have a 70% risk of developing a colon cancer and a 40% risk of developing endometrial cancer. Risk begins to increase after the age of 20-25 years. The tumours arbour microsatellite instability (MSI) and usually loose MLH1, MSH2 or MSH6 expression.

We report a sporadic case of an eighteen-year-old woman with a poorly differentiated pT4N1M1 colon adenocarcinoma. No familial history could be documented up to now, but the patient's family lives in Africa. The adenocarcinoma was MSI-high and a triple loss of staining of MLH1, MSH2 and MSH6 was observed. Two germline mutations were found: a nonsense mutation in MLH1, and a large duplication of exons 7 to 10 in MSH6. No mutation was found in MSH2.

The very young age of diagnosis in this case could be explained by this double alteration. The loss of staining of both MSH6 and MSH2 might be caused by destabilization of MSH2 because of lack of its partner MSH6.

To our knowledge, this is the first description of digenism in Lynch syndrome. Inheritance's risks in first degree relatives are dramatically increased in comparaison to a family with a single alteration in one gene. Our finding suggests that early case of Lynch syndrome should be investigated for all 3 MMR genes, even if one mutation was already found.

P0548. Mutation analysis of the MSH6 gene in 52 MLH1/MSH2-negative, HNPCC suspect Italian patients

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The HNPCC syndrome bears high cancer risk at colorectum, endometrium and other organs, due to MMR defects. Testing in clinical contexts is frequently limited to MLH1/MSH2, since only a minority of cases are thought to depend on MSH6 and other genes. Within a program aimed to prevent inherited cancer in the Piedmont area, we have implemented the mutation analysis of MSH6 on a set of 88 suspect HNPCC probands, in which 52 were previously found to be MLH1 and MSH2 mut- negative, with the aim to define proper criteria for MSH6 testing. Cases were included on the basis of recognized clinical criteria and on preliminary analyses on the tumor for IHC expression of MMR proteins and microsatellite instability. All 10 MSH6 exons were amplified in 19 amplimers from leukocyte DNA, and analysed by DHPLC. Screening for major deletions was performed by MLPA. Two point mutations (K537fs and Y1286delinsIN), one deletion of exons 2-4 and two variants of unknown pathogenic significance were found. In all three MSH6-mut patients the tumor was MSI-H; in one of them we demonstrated a double-negative IHC pattern (MSH2- and MSH6-). Within the limits of our small sample, MSH6-mut patients showed less typical family history. The inclusion of MSH6 in HNPCC genetic testing can significantly improve sensitivity. A MSH6 and/or MSH2 negative IHC, better than any clinical feature, appears to efficiently pinpoint cases for MSH6 screening among MSH2-neg cases.

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P0549. Combined effect of the *p53* Arg72Pro and the *RNASEL* Arg462Gln sequence variant on the age of disease onset in Hereditary Nonpolyposis Colorectal Cancer (HNPCC) patients

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Introduction: The tumour suppressor gene *p53* plays a key role in the apoptotic pathway, and the prostate-cancer-susceptibility gene *RNASEL* is a tumour suppressor also involved in apoptosis. Recently, we could show that functionally different variants in both genes (Arg72Pro in *p53* and Arg462Gln in *RNASEL*) are associated with the age of onset (AO) in HNPCC patients. Hereby we aimed to assess the combined effect of both variants.

Methods: We screened 246 unrelated HNPCC patients with pathogenic germline mutations in *MSH2* or *MLH1* and colorectal carcinoma as first tumour and 245 healthy controls.

Results: The global log-rank test revealed significant differences in the AO for both variants independently (p=0.0176 for *p53* and p=0.0358 for *RNASEL*) and for the combined genotype of both variants (p=0.0174). The highest difference in median AO was twelve years between homozygous carriers of the wild-type in both genes (42 years) and of the polymorphic allele in both genes (30 years), respectively. Multivariate Cox regression model indicated that the *p53* and *RNASEL* genotypes had a significant influence on AO (p=0.016 for *p53* and p=0.014 for *RNASEL*) in an additive mode of inheritance, and that the effects of both variants are additive without any interaction.

Conclusions: Our results suggest that *p53* codon 72 and *RNASEL* codon 462 genotypes are independently associated with AO in HNPCC in a dose-dependent manner, and that the combined effect is purely additive. This may be relevant for preventive strategies. Our results support the notion that the p53- and Rnase L-pathways do not interact.

P0550. Using quantitative Real Time PCR is a good toll to detect hTERT activity and prognosis of lung cancer

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Telomerase is a ribonucleoprotein enzyme that synthesizes telomeres after cell division and maintains chromosomal length and stability. Approximately 85-90% of human cancers, including lung cancer, show high activity of telomerase. However, the correlation between the quantitatively measurement of telomerase activity and the clinical characteristics of lung cancer patients remains unclear. The aim of this study was to elucidate the clinicopathological relationship between telomerase activity and telomerase reverse transcriptase subunit (hTERT) status in non small cell lung cancer using Real Time Polymerase Chain Reaction (RT-PCR) assay.

RNA was extracted from 23 lung cancer, 23 adjacent normal lung tissue specimens and theirs blood plasmas obtained from patients who underwent surgery. hTERT mRNA expression was estimated by quantitative RT-PCR using LightCycler Instrument.

Clinical and pathologic parameters were evaluated with respect to the level of telomerase activity. Prominent telomerase activity was detected in 19 (82,6 %) lung cancer tissues and 4 (7,4 %) adjacent non-neoplastic samples. In contrast, no telomerase activity was detected in blood serum of these patients.

We observed high correlation with the size of tumor and lymph node metastasis in tissue of tumor, but did not correlate with tumor differentiation and tumor cell type in that patients who underwent operation (p<0.05).

In addition we observed that a higher recurrences rate in that patients who had if the telomerase activity level was higher than the other patients in the same group. These results suggest that the level of telomerase activity is positively correlated with the pathological stage and mortality of lung cancer.

P0551. HumARA CAG polymorphism in human colon cancer

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The HumARA CAG repeat is located in the first exon of Androgen Receptor (AR) coding for a polyglutamine stretch. It is hypothesized that the shorter the length of this tract, the greater the affinity of androgens to the AR and the greater the androgenic effect.

Normal polymorphic tract of CAG varies according to the ethnicity in a range of 8-36 repeats. The CAG expansion over 40 repeats leads to spinal bulbar muscular atrophy (SBMA).

Because of the HumARA marker is highly polymorphic in the normal population up to now it has been commonly used in forensic application, but recently its relationship with cancer risk induced by the length of polyglutamine tract in the AR, suggests to consider it a not suitable marker for forensic casework. A mutation pathway independent of microsatellite instability was delineate in colon cancer CAG alterations suggesting that androgens contribute to colon carcinogenesis.

We have analyzed the HumARA CAG polymorphism in 50 colon cancers in comparison to the surrounding healthy tissue. The samples were previously typed for 15 STR markers used in forensic genetic profiling showing different percentage of MSI-H, and MSI-L.

AR-CAG alterations occur in cancers with and without MSI and no correlation to sex and staging has been demonstrated.

The data seem to confirm the previously report that the AR is an additional target of mutations related to genetic instability and HumARA marker should be replaced by other X-STR markers for forensic purposes.

P0552. Interleukin-10 genotypes in sporadic colon cancer

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Interleukin-10 (IL-10) is an anti-inflammatory and immuno-suppressive cytokine involved in angiogenesis in various cancers. IL-10 production is influenced by single nucleotide polymorphisms (SNPs) in the promoter region of the gene.

We examined IL-10 haplotypes consisting of three SNPs -1082(G/A), -819(C/T) and -592(C/A) within the promoter region of the IL-10 gene. IL-10 production is associated with haplotypes that are defined by these SNPs, so that individuals can be genotyped for higher or lower levels IL10 production. These haplotypes might influence the risk for colon cancer development and progression possibly by affecting the efficiency of the antitumour immune response and/or pathways of angiogenesis.

A total of 100 sporadic colon cancer (SCC) patients and 100 unrelated cancer-free controls were genotyped for -1082 SNP using real-time PCR TaqMan® SNP genotyping assay. PCR-RFLP method was used for -819 and -592 SNPs.

The most frequent IL-10 genotypes in both groups were medium expressing genotypes ACC/GCC (30,0% in control and 29,9% in SCC) and ATA/GCC (22,9% in control and 24,7% in SCC). High producing genotype GCC/GCC was detected in 17,1 % of controls, and 15,5% of SCC samples. Low producing genotype ACC/ACC was more frequent in controls (20,0%) when compared to SCC (6,2%), and low producing genotype ATA/ATA was more frequent in SCC (8,2%) when

compared to the control where it was not detected at all. Combined genotype ACC/ATA was detected in 10,0% of control and 15,5% of SCC samples.
Our results indicate that some of the specific IL-10 genotypes might be associated with sporadic colon cancer.

P0553. Molecular and immunohistological properties of juvenile polyps from *SMAD4* and *BMPR1A* mutation carriers

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Juvenile polyps are the most frequent type of colorectal polyps in children and adolescents. In general, these lesions are solitary and have a low potential to become malignant. However, in patients affected by the autosomal dominantly inherited juvenile polyposis syndrome (JPS), multiple juvenile polyps are developed throughout the gastrointestinal (GI) tract which if left untreated may cause bleeding, anemia and carry an elevated risk of developing GI cancer. Germline mutations in the *SMAD4* or *BMPR1A* genes, the members of the transforming growth factor beta (TGFβ)-receptor SMAD superfamily, have been shown to cause disease in 35-50% of JPS patients. So far, little data is available on the nature of the second, somatic mutation (eg. loss of heterozygosity (LOH)) and oncogene activation in tumours from JPS patients with an identified germline mutation. Here we present the results of a molecular genetic survey on two juvenile polyposis kindreds harbouring pathogenic *SMAD4* (1244-1247delACAG) and *BMPR1A* (583C>T; G195X) germ line mutations, respectively. Twenty-four tumour specimens from mutation-positive family members were assessed for the presence of LOH at the *SMAD4*, *BMPR1A* and *APC* gene loci, the frequency of somatic mutations in the *KRAS*, *BRAF* and *p53* genes as well as SMAD4 expression and tissue localisation. Based on these findings, the different molecular genetic events and pathways involved in *SMAD4* / *BMPR1A* tumourigenesis will be discussed.

P0554. Evaluation of genetic damage due to chemotherapy and radiotherapy in patients with Acute Lymphoblastic Leukemia by micronuclei assay

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Leukemia is a heterogenous group of neoplastic disorder which derived from malignant transformation of hematopoietic progenitors. In most cases leukemia is associated with cytogenetics disorder. In addition cytogenetics disorders are induced by chemical and radiation which are used as therapeutically regimens. Micronuclei assay is one of the sensitive methods in evaluation of genetic and chromosomal aberration. In the present study, genetic alteration due to radiotherapy and chemotherapy protocols in patients with Acute Lymphoblastic Leukemia (ALL) is evaluated by micronuclei assay techniques.
Total of 40 patients with ALL and 10 controls were used as sample subjects. According to treatment phases, patients were divided are into four groups as a without treatment, remission induction, consolidation and maintenance groups. Genetic alterations in these groups were evaluated by micronuclei assay techniques.
The frequency of micronuclei observed for each group was significantly higher than control group. Data also indicate that there were significant differences in the frequency of micronuclei among four groups.
The finding indicate that radiotherapy and chemotherapy in ALL patients mainly kills cells by induction of genetic loss(chromosomal aberration). observation of genetic loss in erytrocytic cells can be considered as a significant side of radiotherapy and chemotherapy.

P0555. A rare complex translocation in a case with cronic myeloid leukemia

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According to literature in 2-10% cases with chronic myeloid leukemia (CML) chimerical gene BCR/ABL ensues on complex translocation, involved together with chromosomes fragments 9q34 and 22q11 one and more other chromosomes.
Here we report on a 20-year old man with chronic myeloid leukemia. FISH analysis has shown a presence of ABL/BCR fusion gene in 100% nuclei. After course of therapy with Glivek (Imatinib mesylate) repeated cytogeneticanalysishasfoundt(2;9?;22).Chromosome9wasapparently normal. Application of LSI ES BCR/ABL probe revealed fusion gene only in 44% nuclei. Using WCP for chromosomes 2 and 22 we detected two cell clones: 46,XY,der(2)t(2;22)(q11;q11)t(9;22)(q34;q12)[16]/46,XY,t(2;22)(q11;q11)[17]. In the first clone presumably occurred two breakpoints on 22q11 and 22q12. The fragment 22q11~22q12 was transferred on der(2)(q11), and other segment 22q12->22qter - on der(9). The terminal part of chromosome 9: 9q34->9qter was detected on der(2) with formation of fusion gene BCR/ABL on der(2). The complex translocation t(2;9;22) was previously found only in two cases with CML.
To the best of our knowledge the prognostic significance of 2q11 segment is not yet described in the literature. Decrease of nuclei number with BCR/ABL fusion gene after Glivek treatment has shown that this variant translocation does not influence on sensitivity of the patient to the drug.

P0556. Complex chromosomal rearrangements of bone marrow cells in three patients with childhood leukemia

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Until now, plenty of chromosomal aberrations have been described as a specific for the particular type of leukemia and they have important diagnostic and prognostic value. Cytogenetic analysis of bone marrow cells in leukemian patients may also, show nonspecific changes of chromosomes. Often, these nonspecific chromosomal aberrations are part of complex karyotype which includes presence of multiple chromosomal aberrations in one bone marrow cell's clone.
In this report we present 3 children with different type of leukemia, diagnosed and treated at Mother and Child Health Care Institute, Belgrade.
First patient was a girl, 15 years old, with diagnosed AML morphology type M1.Cytogenetic analysis of bone marrow cells shown complex karyotype: 46,XX,t(4;10)(q21;q24),del(11)(q23) Patient relapsed in short period and after bone marrow transplantation died.
Second child was a boy, ANLL was diagnosed at the age of 6 and two cell's clones were detected in karyotype of bone marrow cells: 46,XY,-18,del(18)(q21),add(5)(p15) [8] / 46,XY [14]. After short relapse, patient died.
Third patient is a girl with AML, M1 morphology type. The disease was diagnosed when she was 4 years old. Cytogenetic analysis of bone marrow cells revealed: 47,XX, i(7)(q11),t(10;11)(p14;q14),+19. Patient achieved complete remission.
The authors will discus the progress and outcome of different type of leukemia with previously mentioned complex chromosomal rearrangements in bone marrow cells.

P0557. Investigation of *MRP*1 gene amplification in patients with acute leukemia

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Multidrug resistance (MDR) is a complex phenomenon in which many different genes regulating drug transport, cellular repair, detoxification and drug metabolism will activate. Nevertheless, up-regulation of multidrug resistance gene (MDR1) and multidrug associated protein gene (MRP1) could be at the basis of the resistance phenotype *in vivo.* Increase in gene copy number and over-expression are two major mechanisms for increasing the activity of these two genes. In most drug resistant cell lines, gene amplification have been detected but the role of gene amplification in inducing MDR phenotype in clinic has not yet been known. We aimed to evaluate MRP1 gene copy

number in leukemic patients by real time PCR. So mrp1 gene copy number in peripheral blood of 30 patients and 10 healthy volunteers determined and compared with *ß-actin* copy number in each sample. As standard we used leukemic CCRF-CEM cell line (drug sensitive) and its drug resistant subline E1000. MRP1 gene copy number in CCRF-CEM was shown to be normal but has been highly increased in E1000. However no sign of gene amplification detected in healthy and patients groups. Our results suggest that increase in MRP1 gene copy number observed in resistant cell lines is not responsible for MRP1 up-regulation *in vivo.*

P0558. Suspected germline TP53 mutation mosaicism in a case of Li-Fraumeni syndrome

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Li-Fraumeni syndrome (LFS) is a rare hereditary predisposition to a broad range of tumours. About 70% of LFS families carry germline TP53 mutations. We present a girl with clinical symptoms typical for LFS (adrenocortical cancer at 1 year and osteosarcoma at 5 years of age) but with no family history of cancer. A point C>T mutation in exon 8 (Arg282Trp) was identified in DNA isolated from her blood lymphocytes. However, the substitution was detected in neither of her parents indicating that the defect could be a de novo mutation. Careful examination of the sequence data showed that the mutated allele might be present in a lower amount compared to the wildtype allele. Repeated sequencing yielded reproducibly the same findings. We compared the sequencing results with a DGGE (Denaturing Gradient Gel Electrophoresis) analysis with DNA from the patient, DNA from another patient with codon 281 mutation, and DNAs from two tumours with somatic codon 282 mutations. The ratios between the alleles as observed on DGGE correlated closely with the ratios from sequence curves. This led us to the notion that this patient could be a mosaic of normal and heterozygous cells, and prompted us to examine the allele ratio in other non-invasively sampled tissues. The results obtained from urine sediment looked similar to that from blood. On the contrary, in buccal cells both alleles seemed to be present in equal amounts. To our knowledge this is a first description of suspected somatic mosaicism in LFS. Supported by grants MSM0021620813 and MZO00064203.

P0559. Data of NSCLC gene expression pilot study

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Cancer is worldwide problem and lung cancer is one of the frequent ones causing ca 1 million deaths every year. Although early stage lung cancer is in most cases surgically curable, about 80% of lung cancer cases due to tumour spread or distant metastases need either radiotherapy, adjuvant or neoadjuvant polychemotherapy. Despite of certain success achieved in the field of combined therapy, the prolonged use of it is limited by developing resistance to drugs and side effects of this treatment.

In everyday lung cancer diagnostics histological classification is used. Accordingly, lung cancer is divided to small cell lung carcinoma (20% of all lung cancers) and non-small cell lung carcinoma (NSCLC, 80% of all lung cancers) including three main groups: squamous cell carcinoma (20-35%), giant cell carcinoma (4.5-15%) and adenocarcinoma (30-50%). Despite of lung cancer histological subgroup diagnostics, the clinical course of the same stage patients is quite different. This fact suggests that histological form of cancer is not sufficient predictor of clinical course of the disease.In the current study we have used cDNA microarrays with 46.000 features to monitor gene expression patterns of different NSCLC samples in hope to find differentially expressed genes that would help us to discriminate different NSCLC types and eventually predict the survival and clinical course of the patients.

P0560. Cytokine SNPs screening analyses in canine malignant histiocytosis

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Malignant histiocytosis is a tumour disease which is characterised by increasing proliferation of macrophages and reinforced degradation of erythrocytes. High progression of this disease leads to an unfavourable prognosis for the patients most of them children up to the age of three years. Neoplastic histiocytes frequently infiltrate bone marrow, spleen, lymph nodes, liver and lung tissues. Histological and cytological findings proposed an important role of aberrant expression of cytokines in histiocytosis. Due to the fact that the Bernese Mountain Dogs show a predisposition for a spontaneously development of malignant histiocytosis these dogs could be used as a genetic model organism to dismantle the mechanisms of human malignant histiocytosis.

Therefore we screened the canine cytokine cDNA transcripts of *TNFα*, *Interleukin-1-alpha* (*IL1A*) *and Interleukin-1-beta* (*IL1B*) for single nucleotide polymorphisms (SNPs). Total RNA was isolated from lung, spleen, testis, and skin tissues of nine different dogs (seven Bernese Mountain dogs, one Collie and one Westhighland Terrier). We amplified and sequenced the corresponding cytokine cDNAs by RT-PCR, screened them for SNPs and analysed the effects caused on the protein sequence.

For *TNFα* one nucleotide insertion in exon 1 coding sequence of one Bernese Mountain Dog individual showed a frame shift mutation leading to a truncated form of TNFα protein. Several Bernese Mountain Dogs and the West Highland Terrier showed SNPs in the coding sequences which lead to missense mutations within the protein sequences of ILA and ILB.

P0561. Study of Correlation of MRP1, and MDR1 Expression and Function in Iranian AML Patients

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A major problem in treating patients with cancer by chemotherapeutic regimes is that their tumors often develop a multidrug resistant (MDR) phenotype and subsequently become insensitive to a wide range of chemotoxic drugs.

Multidrug resistance can result from changes that limit accumulation of drugs within cells by limiting uptake, enhancing efflux, or affecting membrane lipids such as ceramide.

ABC (ATP-binding cassette) transporters are a family of transmembrane proteins that can transport a wide variety of substrates across biological membranes in an energy dependent manner. Their overexpression is associated with increased efflux of chemotherapeutic drugs.

The MDR1 gene is located on chromosome 7q21.12 ,encodes for P-gp. P-gp transports many hydrophobic substrates and anti-cancer drugs including etoposide, doxorubicin and vinblastine.

The MRP1 (ABCC1) gene is located on chromosome 16p13.12. MRP1 can mediate the transport of negatively charged conjugated hydrophilic compounds with a large hydrophobic moiety such as glutathione *S*-, glucuronide, and sulfate conjugates of drugs. It can extrude neutral and basic organic compounds if the cell contains normal levels of GSH, probably by co-transport of the drug with GSH.

In a preliminary study using RT- real time PCR we found corelationship between increase MRP1 expression level and resistance to treatment among patients with AML. Now we aim to detect and measure the MRP1 protein in this group of AML patients.

Furthermore, we want to investigate the possible role of other reported gene such as MDR1 among AML patients with MDR phenotype for whom high level of MRP1 expression could not be detected.

P0562. MYBL2, ZNF217, CYP24 and STK6 gene amplifications are rare events in melanoma tumorigenesis

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Comparative genomic hybridization on paired primary and metastatic melanoma identified a relation between chromosome 20q abnormalities and melanoma progression. The purpose of this study is to determine the frequency of gene copy number changes on chromosome 20q and to evaluate their significance for melanoma progression.

Fluorescent in situ hybridization with four BAC clones corresponding to *MYBL2*, *ZNF217*, *CYP24* and *STK6* genes (located at chromosomal region 20q13.1-q13.2) was applied on tissue microarray, consisting of 280 primary melanomas and melanoma metastases. A BAC probe corresponding to centromere 20 was used as a control.

The study showed low level of amplification ranging from 1.17% to 2.03%. The frequency of gain varies between 5.41-7.63% and involves single gene or more genes per sample. Amplification occurs in two subamplicons, since coamplification between *ZNF217*, *CYP24* and *STK6*, separately from *MYBL2* gene changes was assessed. Additional copies of centromere 20 were detected in 28.34%. A significant difference was observed between primary tumors and metastases regarding the frequency of aneuploidy of chromosome 20 (p=0.05). The more progressed the tumor was, the higher an increase in centromere copy number was detected.

In conclusion, aneuploidy of chromosome 20, rather than gene specific copy number changes of *MYBL2, ZNF217, CYP24* and *STK6* is the mechanism involved in melanoma progression.

P0563. Methyl Primer Express® Software and influence of amplicon characteristics to successrate in sequencing of bisulfite treated DNA

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A well known method to study methylation patterns is to treat gDNA by sodium bisulfite to distinguish methylated cytosine (5mC) from unmethylated C, which is deaminated to uracil (U) and replaced by thymine (T) in subsequent amplification. 5mC still remains as C. Subsequent amplification can focus on selective amplification of methylation patterns in CpG islands (methyation specific PCR, MSP) or on amplification of bisulfite treated (converted) gDNA (Bisulfite treatment specific PCR, BIS). Selection of PCR focus is done by primer design. After PCR, sequencing can clarify the methylation pattern but several factors must be taken into account to ensure reliable data.

During the bisulfite treatment base composition will undergo dramatical changes. This must be taken under consideration during primer and amplicon design for the initial amplification. Dependend on the base composition of the target region the design may change the originally focused strategy for sequencing.

In first instance, strategy is depending on preferred outcome:

1. More or less methylated? / Which CpG in target region is differentially methylated? > Direct sequencing of PCR products.
2. Semi-quantitative results > Cloning of PCR products and sequencing of multiple clones.

In a second instance, the base composition of the amplicon itself will lead to the conclusion, whether direct sequencing of PCR products can be done or not.

Here we use a new PC Software called “Methyl Primer Express®” to design BIS oligos on different promotor-target regions to show examples for critical amplicons and recommendations for successful amplification and sequencing.

P0564. Methylenetetrahydrofolate reductase C677T polymorphism and colorectal cancer risk in Republic of Macedonia

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Polymorphisms in genes involved in the metabolism of folate and methyl groups have been implicated with risk of colorectal cancer. 5,10-methylenetetrahydrofolate reductase (MTHFR) plays a central role in folate metabolism, irreversibly converting 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which is a vital source of methyl groups for DNA methylation. A C ->T polymorphism at nt.677 in egzon 4 of the MTHRF gene was described which results in enzyme variant with reduced activity of 30% in heterozygotes and up to 70% in homozygotes. We evaluated the relation between this polymorphisms and risk of colorectal cancer in a case-control study involving 115 patients with colorectal cancer and 111 controls from the Republic of Macedonia. Genotypes were obtained by PCR-RFLP analysis on DNA samples isolated from peripheral blood. Risk of colorectal cancer was estimated with conditional and unconditional logistic regression. Relative risk estimates were similar between patients and controls irrespective of the sex, age, Dukes stage, family history or type of genetic instability (MSI or CIN) of tumors of the patients. Higher frequency of T-allele was found only in patients with tumors in the upper colon (0.54 vs. 0.34, p=0.05, 95% CI: 0.9872<R.R.<3.9778) resulting in ~3 fold reduction of the relative risk for this type of colon cancer in homozygotes. The protective role of MTHFR 677Tallele with low potential for DNA methylation for the development of rightsided colorectal tumors is a new observation which should be confirmed in a larger cohort of patients with different ethnic background.

P0565. Germline mutation of both copies of the *MLH1* gene could play an important role in the early-onset of CRC

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Objectives: Hereditary nonpolyposis colon cancer (HNPCC) is an autosomal dominant inherited disorder that is characterized primarily by the development of colorectal cancer (CRC). CRC in adolescent patients is extremely rare even in families with HNPCC. Those cancers require particular attention because the survival rate for this young group of patients is poor. Here, we describe a family with HNPCC and two germline mutations in *MLH1* gene and very early onset colorectal cancer in the proband (12 years old). Our observations suggest that the germline mutation of the both copies of the *MLH1* gene could play a important role in the early-onset of CRC.

Study design: Genomic DNA was isolated from peripheral blood lymphocytes from the proband and 8 members of the HNPCC family and sequenced for *MLH1* and *MSH2* genes. DNA obtained from proband´s sigmoid tumour specimen was analyzed for high frequency microsatellite instability (MSI).

Results: Two germline *MLH1* gene mutations have been identified. One located in the exon 8 of *MLH1* gene, R226X, and other change located in the exon 19 of *MLH1* gene, V716M. Proband’s tumor demonstrated MSI at each loci tested, defining the tumor as having high-frequency MSI.

Conclusions: In this patient, V716M mutation in addition to R226X may lead to inactivation of both copies of the *MLH1* gene allowing earlier accumulation of mutations in microsatellites and other repetitive sequences, generating MSI and initiating the tumorigenic process.

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P0566. Evaluation of 11q23 rearrangements in Turkish ALL and AML patients by FISH

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Structural abnormality of 11q23, including the MLL gene, is a recurrent chromosome change observed in 3-7% of acute lymphoblastic leukemias (ALL) and in 3-4% of acute myeloblastic leukemias (AML). Although over 30 variant translocations are known to be related to MLL translocation, the most common abnormalities are t(4;11)(q21;q23),

t(9;11)(p22;q23), t(11;19)(q23;p13) and t(6;11)(q27;q23). Also deletion of 11q23 region is a rare chromosomal abnormality that can be seen in ALL cases. To reveal the 11q23/MLL translocation, we analyzed 52 patients with ALL(45 pediatric) and AML (4 pediatric, 3 adult) using both conventional cytogenetic (CC) analysis and FISH by using LSI MLL Dual Color, Break Apart Rearrangement probe, placing an emphasis on the result discrepancies. Six of the 45 pediatric ALL patients (13,3%) and 4 AML (2 pediatric, 2 adult) patients had the 11q23/MLL translocation, while monosomy 11 was observed in 1 ALL patient, and multiple copies of MLL gene was revealed in 1 AML patient and 1 ALL patient. 11q23 rearrangement was detected only in 8 of the 13 patients by FISH. t(4;11) and t(6;11) translocations were detected by both metaphase, FISH and CC analysis in AML patients. Also in 5 patients, chromosomal abnormalities other than 11q23/MLL translocation, was restricted to CC analysis only. Two ALL cases and two AML patients with 11q23/MLL translocation died, the other cases with MLL gene rearrangement are being followed up. As a result, we recommend that 11q23/MLL FISH should be performed in the diagnosis and monitoring of ALL and AML in combination with CC.

P0567. Molecular mechanisms of multidrug resistance in cancer chemotherapy: Clinical significance of MRP1 gene In Iranian Leukemic pateints

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Multidrug resistance (MDR) is one of the main obstacles in treatment of cancer patients. Therefore it has been studied for half a century now, ever since cytotoxic drugs were first used for cancer therapy. So far, about three separate forms of MDR have been characterized in more detail: classical MDR, non-Pgp MDR and atypical MDR. The classical MDR drug pump is composed of a transmembrane glycoprotein (P-glyco-protein-Pgp) encoded by the so-called multidrug resistance (MDR1) gene. Typically, non-Pgp MDR has no P-gly-coprotein expression, yet has about the same cross-resistance pattern as classical MDR. This non-Pgp MDR phenotype is caused by over-expression of the multidrug resistance-associated protein (MRP1) gene.

To the best of our knowledge (although one of the main obstacles in chemotherapy success rate) no one systematically investigated the role of MRP1 gene in inducing MDR phenotype in Iranian leukemic patients. By employing chromosome microdissection we shown that increased in gene copy number and also over-expression of MRP1 gene are involved in drug resistance in a drug resistance leukemia cell line. In addition, we have used fluorescent in situ hybridization (FISH) and Real-Time PCR technology to study the association between MRP1 and MDR phenotype in Iranian AML patients. So far we found that over-expression of MRP1 occurs in about 20% of the Iranian AML patients. We are aiming to further investigate whether or not elevated MRP expression in Iranian patients at diagnosis is an unfavorable prognostic factor for clinical outcome of chemotherapy.

P0568. The importance of functional testing in the genetic assessment of Muir-Torre syndrome, a clinical subphenotype of HNPCC

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A majority of families with hereditary nonpolyposis colorectal cancer (HNPCC) are attributable to germline mutations in DNA mismatch repair (MMR) genes. However, the clinical phenotype of HNPCC patients reflects a complex interplay between the predisposing mutation and putative constitutional and somatic modifiers.

Certain MMR gene mutations predispose to combined occurrence of sebaceous gland neoplasms and visceral malignancies. This is known as Muir-Torre syndrome (MTS), and regarded as a phenotypic variant of HNPCC.

The sebaceous tumors associated with MTS appear in many patients before visceral malignancies, providing important predictability of HNPCC-related cancers in mutation carriers. However, molecular assessment needs to be carried out to verify the presence of the syndrome. Contribution of non-truncating mutations found in skin cancer patients is difficult to interpret and therefore, genetic assessment of MTS requires a functional test.

Here, we studied the repair efficiency of the two MSH2 missense mutations, L187P and C697F, found in HNPCC families including mutation carriers with sebaceous skin tumors. We assessed their capability to correct DNA mispairs with a functional mismatch repair assay. Both mutations were deficient in the assay, which together with tumor findings, high MSI and loss of MSH2 protein expression, suggested their predisposing role in both internal and skin malignancies in the families.

The functional testing of the two missense mutations in MSH2 supports the notion that Muir-Torre subphenotype of HNPCC is associated with severe functional defects in the repair capability of the mutated proteins, which is compatible with a phenotype fulfilling the Amsterdam criteria in the families.

P0569. Characterisation of a new variant of the *MSH2* gene (c.1022T>C - p.Leu341Pro) in 9 HNPCC families of North of France

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Hereditary nonpolyposis colorectal cancer (HNPCC) accounts for ~2% of all colorectal cancer (CRC) cases. Studies at the PAFNORD registry (regional multidisciplinary center of North of France) have revealed a unique germline *MSH2* c.1022T>C-p.Leu341Pro missense mutation that has never been reported. Detailed analyses showed that this specific mutation constituted ~20% of all germline MMR gene mutations identified in PAFNORD registry.

The objectives of this study were : *i)* to determine if the c.1022T>C is the disease causing mutation and not a clinically silent mutation ; *ii)* to characterise the founder effect.

Complete analysis of *MSH2, MLH1* and *MSH6* did not reveal other pathogenic mutations. The c.1022T>C variant was identified in 7.1% of HNPCC families in PAFNORD registry and was not identified in 103 healthy controls. All colorectal cancer tested showed microsatellite instability (4/4) and absence of MSH2 protein (3/3), by immunohistochemical analysis. The variant is located in a conserved domain of MSH2 important for structure of the protein. The c.1022T>C variant segregates with the HNPCC phenotype in 17 patients (lod-scores are being performed).

Thi allele was identified in 9 apparently unrelated families. All originated from the french region of Lille in North of France. Haplotypes are being performed using microsatellite markers flanking the *MSH2* gene in order to characterise this variant as a founder mutation in North of France.

All these results support the hypothesis that the c.1022T>C variant is the disease causing mutation and encourage us to use it in presymptomatic screening, even if functionnal assay has not been performed.

P0570. The prevalence of a germline *MSH6* mutation is very low in patients without microsatellite instability in their tumour

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Introduction: Germline mutations in the *MSH6* mismatch repair gene account for a minor part of Hereditary Non-Polyposis Colorectal Cancer syndrome (HNPCC). Failure of the DNA mismatch repair system causes high level microsatellite instability (MSI-high) in tumours of HNPCC patients and MSI analysis is often used to start

HNPCC diagnosis. The sensitivity of MSI analysis to detect *MSH6* mutations is questioned.
Methods: To investigate the role of *MSH6* mutations in a cohort of HNPCC suspected patients without microsatellite instability in their tumour DNA, we performed immunohistochemical staining of the MSH6 protein in these tumours and analyzed the *MSH6* gene in patients most suspected of HNPCC.
Results: All 380 tumours that were not MSI-high showed presence of the MSH6 protein. In 90 patients, from this group of 380 patients, *MSH6* germline mutation analysis was performed, and no pathogenic mutation in *MSH6* was detected. Pathogenic *MSH6* mutations were exclusively found in patients with MSI-high tumours with loss of *MSH6* expression (n=9) and in 2 patients in which MSI analysis was not performed.
Conclusion: Presence of MSI-high in tumour DNA is reliable to select patients for *MSH6* germline mutation analysis. The prevalence of a germline *MSH6* mutation is very low in patients with tumours that are not MSI-high.

P0571. MSH6 gene analysis in patients with Lynch syndrome

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Lynch syndrome (hereditary non-polyposis colorectal cancer) is a cancer susceptibility syndrome with autosomal dominant inheritance and high penetrance. It is caused by germline mutations in mismatch-repair genes, predominantly MLH1, MSH2 and MSH6. MSH6 mutations account for about 7% of all germline mutations in mismatch-repair genes.
We screened for MSH6 gene defects in 59 patients with high predisposition to cancer and without MSH2 or MLH1 mutations. Fifteen of these patients fulfilled the Amsterdam criteria. We used PCR-based DGGE and sequencing to detect point mutations and MLPA (Multiplex Ligation-dependent Probe Amplification) to detect genomic rearrangements of the MSH6 gene.
We detected 14 different variants. Two of them were clearly pathogenic - Ser601Stop and c.1135_1139delAGAGA. Four were intronic substitutions, five synonymous substitutions and three were missense variants. One of the latter, Arg1076Cys has not been published yet. It is located in a conserved domain of the MUTS proteins and causes a non-conservative amino acid substitution. We found this variant in one from 110 population-based controls. The same result was obtained with the Val878Ala variant, which had been considered as possibly pathogenic.
We did not detect any rearrangements of the MSH6 gene in any of the 59 patients.
We can conclude that the frequency of MSH6 mutations in our series is lower than expected and the pathogenicity of some variants is not clear.
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P0572. Molecular diagnostics of multiple endocrine neoplasia type 2 (MEN II) in Slovenia

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Genetic testing for the RET-protooncogene germline mutation showed almost 100% sensitivity and specificity for identifying those at risk of developing inherited medullary thyroid cancer (multiple endocrine neoplasia (MEN) 2A, MEN 2B, or familial medullary thyroid carcinoma (FMTC). Presymptomatic detection of mutations and prophylactic surgical intervention are now the accepted standard of care. In 1996 we introduced a proposal for genetic screening of Slovenian patients with the hereditary form of MTC, and their relatives. Consequently, we have to date identified by genetic analysis 30 carriers of the RET protooncogene mutation among 145 Slovenian MTC patients and their relatives; 15 with codon 634, 9 with codon 618, 5 with codon 790 and one with codon 918 mutation of the RET oncogene, respectively. Genotype - phenotype correlation revealed MEN 2A in 15 patients, FMT in 14 patients and the MEN 2B phenotype in one patient, respectively. The standard treatment was total thyroidectomy, which was performed in 28 patients, while 2 patients rejected any therapy. Use of genetic assay allows earlier and more definite identification and clinical management of those with a familial risk of medullary thyroid cancer in comparison to the standard monitoring used previously.

P0573. Evaluation of 13q14 deletions by interphase FISH in Multiple Myeloma and Chronic Lymphocytic Leukemia Patients

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Multiple Myeloma (MM) and Chronic Lymphocytic Leukemia (CLL) are two of the most common hematological malignancies in adults originating from B-lymphocytes. 13q14 deletions/monosomy 13 have been observed with a frequency of 40-60% and 34% in CLL and MM respectively. Deletions at 13q14 are associated with unfavorable prognosis in patients with MM whereas associated with favorable outcome in CLL cases. We evaluated 13q14 deletions in 19 MM and in 5 CLL cases at diagnosis by interphase FISH combined with conventional cytogenetics (CC) as both diseases have a low proliferative rate making CC analysis difficult. CC studies revealed informative karyotypes in 14 of the 19 MM cases (73.7%). Chromosomal abnormalities were detected in 7 of 19 MM cases (36.8%) including three cases with monosomy 13 associated with complex karyotypes by CC. 13q14 deletions were detected by FISH in 5 of the MM cases (26.3%). These deletions presented as monoallelic 13q14.3 deletion in one and monosomy 13 in 4 MM cases. In this study in addition to the CC, application of FISH studies for 13q14 deletion was increased the number of the cases with chromosomal abnormality up to 47.3% in MM. We could not obtain informative karyotypes from CLL cases. while 13q14 deletions were obseved in 4 of the CLL (80%) cases by FISH analysis. Deletion was monoallelic in three CLL cases, and biallelic in the fourth case. Our preliminary results show that interphase FISH application in conduction with CC studies increase the chromosomal abnormality detection rates both in MM and CLL cases.

P0574. New genetic variants of the *MYH* gene in *APC* mutation-negative patients from Galicia (NW Spain)

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Germline mutations in the base-excision-repair gene, *MYH*, have been associated with recesive inheritance of multiple colorectal adenomas and cancer. In contrast, germline mutations in the *APC* gene have been shown to cause the dominant inherited colorectal cancer syndromes of classical familial adenomatous polyposis (FAP) and attenuated familial adenomatous polyposis (AFAP).
Thirty-one unrelated *APC* mutation-negative Galician patients with either classical (>100 colorectal adenomas) or attenuated (<100 colorectal adenomas) polyposis were screened for germline mutations in *MYH*. Direct DNA sequencing of the entire coding region and the adjacent intronic sequences of *MYH* was performed.
Three (9.7%) biallelic and three (9.7%) monoallelic *MYH* germline mutations carriers were identified in five AFAP and one FAP families. The two most frequent germline mutations (p.Y165C and p.G382D) were observed. In contrast to other populations where the frequency of these two mutations is quite similar, G382D was the most common mutation in galician patients (56% *vs* 11%). A novel mutation p.Q388X (c.1162 C>T) and other new variants in either exonic or intronic sequences were described (GenBank reference sequence NM_012222.1).
Monoallelic *MYH* mutation carriers may be at an increased risk for developing colorectal cancer. However further studies are required to establish a correlation of monoallelic mutations with the disease.

P0575. Mutation analysis of the *MYH* gene in Unrelated Czech APC mutation-negative polyposis patients

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In 20-30% of patients with classical familial adenomatous polyposis (FAP) and up to 90% of those with attenuated polyposis (<100 colorectal adenomas; AFAP), no pathogenic germline mutation in the adenomatous polyposis coli (*APC*) gene can be identified. Some of the *APC* negative FAP and AFAP cases have recently been found to be attributable to *MYH* associated polyposis (MAP). MAP is an autosomal recessive syndrome associated with 5-100 colorectal adenomas and caused by mutation in the *MYH* gene. The MYH protein plays an important role in the base-excision-repair system as an adenine-specific DNA glycosylase.

We screened for germline *MYH* mutations in 90 *APC*-mutation-negative probands with classical and attenuated familial adenomatous polyposis. The entire coding region and intron-exon borders of the *MYH* gene were analyzed. As a prescreening to detect DNA sequence changes, denaturing high performance liquid chromatography (dHPLC) was performed using the WAVE nucleic acid fragment analysis system (Transgenomic). Samples showing unique profiles were sequenced in both directions on ABI Prism 310 Genetic Analyzer (Applied Biosystems).

In majority of patients multiple genetic changes in *MYH* gene were found. Altogether 10 previously reported changes and 8 novel genetic alterations, mostly in intronic sequences were identified. We have detected compound heterozygotes for two the most common germline mutations c.494A>G (p.Y165C); c.1145G>A (p.G382D) too. These variants are established to be associated with adenomatous polyposis and colorectal cancer.

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P0576. Expression of putative NF1 modifying genes in neurofibromas

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Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominantly inherited tumor diseases. Typical hallmark symptoms are multiple neurofibromas, hyperpigmented areas of the skin (café au lait spots) and Lisch nodules. As the symptoms of NF1 show a high interfamilial variability, the existence of modifying genes has long been proposed. NF1 patients with microdeletions spanning the *NF1* and several surrounding genes with yet unknown function have an earlier onset of neurofibromas and a higher tumor load as classical NF1 patients. Therefore, it seems likely that one of the genes additionally missing in these patients contributes to the severity of the disease. On the other hand, two variants of classical NF1 exist where patients present with markedly fewer or no neurofibromas. Familial Spinal NF (FSNF) is characterised by the presence of spinal neurofibromas with a wide and symmetric distribution. Patients with this disease generally lack dermal neurofibromas. Neurofibromatosis-Noonan syndrome (NFNS) is a variant showing phenotypic overlap with Noonan syndrome (including short stature, facial anomalies and webbed neck). It was recently shown to be caused by mutations in the *NF1* but not the *PTPN11* gene which is responsible for plain Noonan syndrome. We are planning a detailed investigation of the 14 genes surrounding the *NF1* gene in patients with FSNF or NFNS compared to patients with classical NF1. We will present data on the expression of these 14 genes in neurofibromas and control tissues.

P0577. New approaches to DNA-diagnostics of neurofibromatosis type 1 in Russian Federation.

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with high index of spontaneous mutations and extremely varied clinical manifestations. The disease is caused by mutations in the tumor suppressor gene *NF1*. Loss of *NF1* gene product (neurofibromin) expression is associated with elevated Ras activity and increased cell proliferation, predisposing to a variety of tumors of the peripheral and central nervous systems. We have developed the protocol for direct and indirect NF1 DNA-diagnostics. The presence in the genome of several unprocessed *NF1* pseudogenes severely hampers mutation analysis of this gene. To overcome these difficulties, we have designed new specific primers for exons 10a, 10b, 11, 19b, 21, 24 and 42. Moreover, we have included additional marker ACint27b in a panel of intragenic microsatellite markers (TCCAint1, D17S1849, TAGA/TAGGint27a and GTint38) that has increased indirect NF1 DNA-diagnostics efficiency. DNA samples from 60 unrelated NF1 patients were screened for the presence of mutations by SSCP, HD and microsatellite analysis; all mobility shifts were sequenced. Eighteen mutations have been found and sixteen of them were described for the first time. The mutational screening has revealed the presence of small deletions and small insertions (65%), missense and nonsense mutations (14%), splice site mutations (16%) and large deletions (5%). These results show reduction in splice site mutations frequency in comparison with other data (25%). This may indicate insufficiency of our methods for identification of splicing defects that induces us to develop a mutations search technique at a cDNA level in future.

P0578. Expression profiling of Neuromedin U and its receptors in cancer cell lines

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Neuromedin U (NMU) is a regulatory peptide known to act via two G-protein coupled receptors: NMUR1 and NMUR2. A recent study revealed NMU may be involved in an autocrine growth loop in acute myeloid leukaemia (AML). Epigenetic silencing of the NMU promoter occurs in head, neck and esophageal squamous cell carcinomas. NMU has also been shown to significantly reduce cellular proliferation, indicating tumour suppressing activity. Expression analyses of the NMU receptors and ligand were carried out on a panel of human cancer cell lines derived from a range of tissues (prostate, breast, bladder, colon, AML). Expression analyses revealed one of the following patterns: expression of the ligand and both receptors, expression of the ligand and one of the receptors, expression of one or both the receptors but not the ligand. Those cell lines lacking NMU expression were examined for promoter hypermethylation. Twelve out of fifteen cell lines examined expressed the NMU ligand and one or both of its receptors. RT112 (bladder), CACO-2 (colon) and P39 (AML) cell lines showed little or no expression of the ligand. Bisulphite modification and DNA sequence analysis revealed methylation of the NMU promoter in each. 5'Aza dC inhibition of methylation in the P39 cells restored NMU expression. The data indicates that NMU may be involved in an autocrine growth loop via NMUR1/NMUR2 in cancer types other than AML. Lack of NMU expression is due to promoter methylation in the cell lines examined.

P0579. A novel mutation in Neurofibromatosis type 1 (NF1)

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Neurofibromatosis type 1 is one of the most autosomal dominant common diseases with an incidience 1:3000 in all ethnic groups. It is a multisystem disorder with the variable clinical features as café au lait spots, Lisch nodules, multiple freckling, neurofibromas or plexiform neurofibromas, optical gliomas, distinct osseous lesions and learning disabilities. Other clinical findings associated with NF1 are high-signal intensity foci on the T2 weighted MR images of the brain. These areas are going to be observed on MRI and data presented.

Various somatic and germinal mutations in tumor-suppressor NF1 gene are causes of the Neurofibromatosis type 1 disease, more than 650 different mutations have been found up to this time.

The family with three clinical affected members have been examined by molecular genetic methods. We have used PCR and DHPLC methods for detection of small insertions, deletions, indels and nucleotide substitutions. The positive results were confirmed by sequencing analysis. The novel mutation c.1_2delATinsCC has been found at all

family members in the start codon NF1 gene.
Mutation probably prevents the start of transcription and causes haploinsufficiency. It consequently leads to the deficit of Neurofibromin protein and to rare phenotype manifestations.
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P0580. Common DNA polymorphisms in thrombophilia-related genes predispose for oral cancer

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Although traditionally oral cancer was thought to be triggered mainly by environmental factors (tobacco or/and alcohol), recently some reports have implicated thrombosis-related factors in its development. Therefore, we investigated whether DNA polymorphisms affecting gene expression and activity of thrombophilia-related factors, are associated with oral oncogenesis. We studied DNA samples isolated from leukocytes of 176 patients with oral cancer and 120 healthy controls of matched sex,age and ethnicity. The prevalence of 8 polymorphisms was analyzed in the groups of patients and controls by allele-specific PCR or RFLP methodologies: *FV* Leiden, prothrombin(*PT*) G20210A,*MTHFR* C677T,*PAI-1* 4G/5G,*GPIa* $C_{807/}$ T_{807},*IL-1β* C3953T,*TNF-α* -G308A, and *TNF-β* G252A. The observed allele and genotype frequencies of *PT* and *TNF-β* were not statistically different between the groups of patients and controls. There was a significant increase of *MTHFR*,*IL-1β* and *GPIa* heterozygotes in the subgroups of patients without family history of cancer and with a positive family history for thrombophilia ($p<0.01$,$p<0.02$ and $p<0.001$ respectively). The 4G and -G308A alleles, which increase expression of *PAI-1* and *TNF-α* genes respectively, were significantly increased in all subgroups of patients in comparison to controls ($p<0.001$). In view of these results, a greater than previously thought percentage of individuals in the general population(15-40%) may have a major or minor predisposition for oral cancer, and may be at risk with or without the involvement of environmental factors (Vairaktaris et al.*Anticancer Res* 2005;*J Cancer Res Clin Oncol* 2006;*Oral Oncol* 2006;*Eur J Surg Oncol* 2006). The ability to routinely detect these polymorphisms may significantly contribute in prevention of oral cancer.

P0581. BRCA1/BRCA2 mutations in primary ovarian cancer patients

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Individuals carrying inactivating germline mutations in the breast and ovarian cancer susceptibility gene BRCA1 and BRCA2 have an increased risk of developing cancer. Germline mutations of the two genes are transmitted in the autosomal dominant fachion and predispose carriers to the development of ovarian and/or breast cancer. We have screened 176 women with primary ovarian cancer for mutations in BRCA1 (185delAG and 5382insC) and BRCA2 (6174delT) gene in using mutagenically separated PCR, single strand confirmation polymorphism (SSCP) analysis followed by sequencing of variant bands. Three germlime alterations were identified: A frameshift mutation (5382insC) was observed in two ovarian cancer patients with familial cancer history. A unique amino acid substitution in exon 20 (G1748S) was seen in two patients and a splice site variant (IVS20+5 A>T) was deteceted in a patient with ovarian cancer. Moreover, a complex alteration (IVS20+5 A>T and G1748S) was also noted in two patients. However, 185delAG and 6174delT mutations were not observed in ovarian cancer subjects. Our preliminary results indicate that BRCA1 gene are involved in some ovarian cancer patients, both with and without a family history, demonstrating the importance of BRCA1/BRCA2 in the development of ovarian cancer patients in Turkish population.

P0582. Analysis of BRCA1 and BRCA2 genes among unselected ovarian cancer cases in Russia.

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Hereditary ovarian cancer in many cases is connected with BRCA1 and BRCA2 mutations.
We found the BRCA1 mutations in 86% families with ovarian cancer only. This frequency was similar to the mutations occurrence among families with ovarian cancer that included breast cancer also (61%). High incidence of the mutations among familial ovarian cancer stimulated us to investigate BRCA1 and BRCA2 mutations on a sample of 53 unselected cases of ovarian cancer at first on Russian sample. The BRCA1 or BRCA2 mutations have 11 women (21%), including 9 BRCA1 mutations (17%) and 2 BRCA2 missense mutations (4%). In BRCA1 spectrum the more frequent was 5382insC mutation (60%). The three other mutations were C61G, 2080delA and 4154delA. The analysis of family history characteristics is performing at the present time.
Single nucleotide polymorphisms E1038G of the BRCA1, N372H and located in the 5'-untranslated region variant 203G/A of the BRCA2 were investigated among patients without mutations and in control sample of 109 women. Significant association of 203AA homozygous variant with ovarian cancer occurrence was revealed (OR=7.3; P=0.004). This variant may be considered as a marker of ovarian cancer.

P0583. Analysis of loss of heterozygosity at chromosome 17 in ovarian carcinomas

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Ovarian carcinoma is the leading cause of death from gynecological malignancies and one of the most common hereditary cancers among female. Specimens from 5 benign tumors, 1 chronical absces, 1 ovarian cyst, 1 borderline tumor, 21 epithelial ovarian cancer, 6 ovarian cancers that are not epithelial by origin and 5 specimens from ovarian epithelial cancer metastasis, were examined for loss of heterozygosity (LOH) at 2 microsatellite loci: TP53 at locus 17p13.1 and D17S855 (*BRCA1*) at locus 17q21. The frequency of LOH at locus 17p13.1 was 30,7% in epithelial ovarian cancers and 25% in metastasis. LOH at the region of *p53* gene was detected in ovarian cyst and borderline tumor, but it was not found in benign tumors and carcinomas that are not epithelial by origin. The frequency of LOH at locus 17q21 was 50% in benign tumors, 38,5% in epithelial ovarian carcinomas, 50% among non epithelial carcinomas and 33,3% in metastasis. In one epithelial ovarian cancer LOH was found at the both locuses of chromosome 17 at the same time. Our results indicate that LOH at the regions of *p53* and *BRCA1* tumor suppressor genes correlate with advanced stages of ovarian carcinoma and poor survival. In some cases of ovarian carcinoma LOH at locuses 17p13.1 and 17q21 occurs later in tumorigenesis, and predominantly in post-menopausal women. Although, allelic loss of one of these genes may be directly associated with malignant transformation *per se*, because it occurs in all histological grades.

P0584. p53 gene mutation analysis in bladder cancer

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p53 is one of the tumor supressor genes and plays an important role in supression of bladder cancer. Recent studies have suggested that p53 mutations are necessary for tumor formation. p53 is the most commonly mutated tumor suppressor in various human cancers, therefore the presence of p53 mutations may help indicate the prognosis for patients with bladder cancer. Understanding the regulation of p53 function in tumor suppression is essential to design new therapeutic strategies to

treat this disease. We investigated the frequency of mutations in exon 5 and 7 of p53 gene in bladder cancer tissue samples.
Tissue samples from bladder cancer patients were used from paraffin-embedded cancer tissues. DNA was isolated from paraffin-embedded bladder cancer tissue samples using "Xylen Cyanol" method. Screening for p53 mutations was done by polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis of exon 5, 6 and 7 of the gene. Enzyme restrictions were performed on 5th exon and on 7th exon using enzymes HaeII and HaeIII, respectively.
This study was performed on paraffin embedded tissue samples from 22 bladder cancer patients. Mutation rate was 45% in 22 cases, whereas mutation rates of exon 5 and exon 7 were found to be 60% and 40%, respectively. The presence of a p53 mutation may aid in appropriate initial staging and subsequent treatment for patients diagnosed with bladder cancer.

P0585. Detection of p53 gene in breast cancer by PCR amd enzyme restrictioin and demonstration of p53 protein in breast tissue with immunohystochemistry

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p53 is one of the tumor supressor genes and plays an important role in supression of breast cancer. Therefore, understanding the regulation of p53 function in tumor suppression is essential to design new therapeutic strategies to treat this disease. We investigated the frequency of mutations in exon 5 and 7 of p53 gene and p53 protein in breast tissue by immunohistochemical analysis.
Screening for p53 mutations was done by PCR-SSCP analysis of exon 5 and 7 of the gene. Enzyme restrictions were performed on 175th codon on 5th exon and 249th codon on 7th exon using enzymes HaeII and HaeIII,respectively. Each of the paraffin-embedded specimens was immunologically stained for the p53 biomarker expression.
Mutation rate of 20 cases was found to be 30%, whereas mutation rates of exon 5 and 7 were found to be 20% and 11,8%, respectively. After immunohystochemical staining, more than 90% p53 staining was achieved in 4 samples; 3% in 1 sample; 1% in 2 samples and 0,5% in 2 samples. No staining was detected in 11 samples. Staining was positive in 3 p53 mutation cases and 6 mutation cases, and staining was negative in 3 mutation cases and 8 mutation negative cases (Fisher's chi-sq test; p>0.05).
The results obtained in these breast carcinoma paraffin-embedded tissues indicate that no clear-cut linear relationship exists between the p53 mutational status and the extent of its respective mRNA and protein expression. Therefore, direct DNA analyses and functional assays remain the only methods for the reliable detection of p53 mutations.

P0586. Detection of Chromosomal Aberrations by FISH, CGH and SKY in Cultured and Noncultured Solid Tumors in Children during 2003 and 2005

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Pediatric tumors are the second main cause of children´s death in developed countries. Contrary to leukemias chromosomal alterations associated with malignant transformations are less well characterized for pediatric solid tumors. We present results of cytogenetic examinations of 262 pediatric solid tumors. Some of them were examined using by interphase fluorescent in situ hybridization (I-FISH), especially tumors where deletions or amplifications of specific genes and/or presence of fusion genes are known and could be used as diagnostic tool. Proven prognostic significance of the N-myc amplification, del 1p36, gain 17q, abnormalities on chromosome 11 are well known for children with neuroblastoma.
For medulloblastomas i(17p), c-myc and i(12p) in germinal tumors are the other candidates. Vast majority of tumor samples were cultured and other possible chromosomal abnormalities were detected using FISH, spectral karyotyping (SKY) and comparative genomic hybridization (CGH). During last few months we have applied also high-resolution comparative genomic hybridization (HR-CGH). Cultivation of solid tumor tissue is not always successful, possible explanation could be generally low proliferation activity of tumor cells in vitro. Here we present our cytogenetic results together with clinical characteristics.
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P0587. Molecular genetic analysis of apparently sporadic pheochromocytomas and paraganglioma in Czech patients

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The pheochromocytoma is 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 nonsyndromic 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 one to pheochromocytoma and paraganglioma. All established genes were analyzed to investigate possible genetic cause of apparently sporadic pheochromocytoma (ASP) and paraganglioma in population of Czech patients. Among 27 patients with ASP two novel germline (missense) mutation was found in the VHL gene. Further, one novel coding single nucleotide change and one inversion in SDHB gene were detected. In a child with neuroblastoma and paraganglioma we detected a novel splice site mutation in SDHB gene. In addition, in one examined patient with apparently sporadic paraganglioma we detected recently identified mutation of the start codon in SDHD gene. These results confirm that molecular genetic testing makes it possible to differentiate the sporadic from the hereditary form of the disease, which will affect medical management.
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P0588. Transforming Growth Factor Beta 1 Leu10Pro polymorphism and breast cancer morbidity: A case-control study and meta-analysis

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Studies have shown that TGF-β_1 has a dual role as a tumor suppressor in early stages and a tumor promoter in later stages of carcinogenesis. In this gene, a Leu10Pro substitution leads to higher circulating levels of TGF-β_1. This variant has been studied in relationship to the risk for breast cancer yielding contradicting results. We aimed to unravel the relationship of this polymorphism and the risk of breast cancer by means of an association study and a meta-analysis.
Women participating in the Rotterdam Study including 163 patients with breast cancer were genotyped for this polymorphism. We carried out a logistic, a survival analysis by age and finally, we performed a meta-analysis.
The logistic regression analysis showed an increased risk of breast cancer for Proline allele carriers (OR=1.5; 95%CI =1.1-2.2) against non-carriers. Which was maintained when studying only incident cases (OR=1.8; 95%CI =1.1-2.8). There were no differences in risk among prevalent cases. The survival analysis showed that carriers of this same allele had a HR of 1.8 (95% CI = 1.2-2.7) compared non-carriers. Finally, the meta-analysis showed the no difference in risk of breast cancer between proline allele carriers and non-carriers (OR pooled = 1.02, 95% CI = 0.95-1.09, heterogeneity = 28%).
Our data suggests that the TGF-β_1 Leu10Pro polymorphism might play a role in breast cancer risk.

P0589. An important cause of polyposis coli: mosaïc *APC* mutations

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Background: The patient with multiple polyps in the colon poses a diagnostic challenge. Given the relatively high frequency of *de novo APC* mutations, a substantial proportion of mosaic *APC* mutations are to be expected in patients with polyposis coli. So far, only four cases of mosaic *APC* mutations have been reported.
Methods: We performed germline mutation analysis in 568 consecutive index-patients with polyposis coli referred for *APC* scanning using DGGE and PTT, and subsequent sequence analysis in DNA fragments displaying abnormal patterns. *APC* gene deletions were detected either with Southern blot analysis or MLPA.
Results: Scrutinizing the molecular genetic results of 223 index-patients with pathogenic *APC* mutations lead to the identification of 11 mosaic cases (5%). Eight mosaic cases were identified as somatic, one case as gonadal and two cases as combined gonadal and somatic. We observed C>T transitions in CGA-sites in four of the eight cases with somatic mosaicism, which is significantly more than 25 of the 212 non-mosaic patients from our cohort (p=0.011). Phenotypes of patients with somatic mosaicism ranged from an attenuated form of polyposis coli to florid polyposis with major extra-colonic manifestations.
Conclusions: Mosaicism accounts for a significant number of *APC* mutations and is predominantly identified in apparently *de novo* cases of FAP. Patients with a somatic mosaicism exert a wide variety in phenotype and arise repeatedly from mutations affecting CGA-sites. These observations have consequences for molecular genetic *APC* testing and the clinical management of polyposis coli patients and their family members.

P0590. Strategies and outcomes of preimplantation genetic diagnosis of familial adenomatous polyposis (FAP)

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More than fifteen years after the first birth after preimplantation genetic diagnosis (PGD), the progress in single cell PCR and mutation detection has led to a considerable increase in the number of available diagnoses. If PGD was initially proposed to couples at risk of having a child affected by a severe genetic disorder, late onset diseases such as Huntington 's disease and, more recently, inherited cancers have become a new class of indications.
We present here our experience of PGD for familial adenomatous polyposis, a dominant inherited cancer due to APC gene mutations. Twelve couples were referred between 2000 and 2005.
We initially developed tests to detect the mutation alone, but rapidly we set up multiplex PCR combining mutation detection and indirect diagnosis using linked microsatellites. Protocols were optimised by modifying standard conditions, particularly after a judicious primer choice, in order to add internal controls in single cell PCR. Finally, we have set up multiplex indirect diagnoses to be able to offer a PGD whatever mutation is involved in familial cases. Mutation detection strategies were based on: (i) a new double allele-specific PCR approach allowing the simultaneous detection of the wild type and mutated allele; (ii) sizing the PCR fragments for deletions; or (iii) restriction length polymorphisms.
We developed 7 tests which were validated on more than 500 single cells. Nine PGD cycles have been performed for 3 couples, resulting in 11 embryo transfers and 3 pregnancies, with the birth of one healthy boy and one ongoing pregnancy.

P0591. Identification of Men with a Genetic Predisposition to Prostate Cancer: Targeted Screening in BRCA 1 / 2 Mutation Carriers and Controls: the IMPACT study.

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We would like to introduce an international screening study aimed at men with a high risk of developing prostate cancer. Population studies show that the risk of prostate cancer may be influenced by heritable factors. Mutations in the *BRCA1/2* genes are known to increase the risk of breast and ovarian cancer and may also increase the relative risk of prostate cancer from 1.5-fold up to 23-fold depending on the gene involved and the context of the mutation.
As prostate cancer is an indolent disease in much of the general population, screening for it is controversial, with no established reduction in mortality. We aim to establish whether male *BRCA1/2* mutation carriers have a higher incidence, are at risk of more aggressive prostate cancer and whether new markers can be identified.
Samples will be taken yearly from 850 *BRCA1/2* mutation carriers and compared with a control group of men who have a negative predictive genetic test over 5 years. This international collaboration will be the largest ever screening programme in men with these mutations. It will also enable us to determine the sensitivity and specificity of PSA screening, research into new disease markers and determine a biological profile of *BRCA1/2* mutation carriers using proteomics and DNA/ RNA microarrays. Our current accrual rate is 100%. Complete recruitment of the UK cohort is anticipated by the end of the year. Baseline PSAs, prevalence of undiagnosed prostate cancer, and the age of onset in male *BRCA1/2* mutation carriers will be compared with the control group.

P0592. AIDIT - expanding familial prostate cancer research in Eastern Europe

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AIDIT (Advancing International Co-operation and Developing Infrastructure for Targeted Screening of Prostate Cancer in Men with Genetic Predisposition) is an international project, funded by the Sixth Framework Programme of the European Community (EC), which aims to facilitate co-operation between European countries in the field of cancer research. AIDIT will focus on linking clinical and research teams in countries new to, or soon to enter, the European Union with the IMPACT Consortium, an international team investigating screening and diagnostic practices for men with a genetic risk of prostate cancer.
Cancer research has been targeted as a high priority for the EC; however, research is most successful when it is centralised and well co-ordinated, avoiding the duplication and fragmentation associated with smaller, isolated studies. AIDIT provides the opportunity for teams who might be new to research in genetics or oncology to become integrated into an existing study. Communication between new and existing collaborators will allow the sharing of knowledge and formulation of strategies to overcome local barriers and challenges.
The AIDIT team will establish and present a website to facilitate communication between project collaborators, and facilitate a conference to bring together international research teams, clinicians and policy makers. The project will also aim to raise awareness of familial prostate cancer among health professionals and the public within Associate Candidate Countries and New Member States of the EU.
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P0593. Expression pattern of androgen metabolism genes support a neuroendocrine differentiation in an androgen-dependent prostate cancer cell line treated by valproic acid (VPA) treatment

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Prostate carcinoma (PCa) originates as an androgen-dependent hyperproliferation of the epithelial cells of the gland and it evolves in an androgen-independent, highly aggressive cancer for which no cure is available to date. It was supported that neuroendocrine differentiation plays an important role in the progression of prostate cancer to androgen independent state.

We studied the effect of Valproic acid (VPA), an inhibitor of hystone deacetylase (HDAC), in a human prostate cancer androgen-dependent cell line (LnCaP).

LnCaP were treated with 5mM VPA, and after 48 hours, assayed for viability, cell proliferation and gene expression patterns by a low-density microarray (AndroChip). VPA was able to induce apoptosis and inhibition of cell growth. The treatment caused a decrease of PSA secretion and stimulated a NSE (neuron specific enolase) expression. Microarray analysis showed that VPA was able to modulate the expression of different androgen metabolism genes and in particular, up-regulation of the UGT2B7 (FC 1.9) gene, involved in DHT (dihydrotestosterone) degradation, and a down-regulation of PSA (FC - 6.19), AR (FC -1.7) and its coregulator ARA24 (FC -2. 89).

The results suggest that VPA treatment reduces the expression of PSA (at RNA and protein levels) and also the amount of androgen available to prostate cells by increasing UGT2B7 expression, nevertheless, the treatment stimulates the production of NSE, down-regulates AR expression, and promotes neuroendocrine differentation. Taken together, these results suggest that VPA treatment should not be considered as a chemotherapeutic agent in androgen-dependent prostate cancer.

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P0594. Quantitative study of mitochondrial RNAs in prostate cancer.

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Prostate cancer (PCa) is one of the commonest cancers in male population around the world, and it is responsible for nearly 6% of all male cancer deaths. Despite this relevance, the mechanisms involved in the development of the prostate malignancy remain unknown.

In the last years there have been several reports of mitochondrial dysfunction in some human cancers. The aim of our group was to perform a quantitative study of the mitochondrial RNA in tumour and non-tumour samples from PCa patients.

We analysed the mitochondrial respiratory chain genes ND2, ND4, ND6, COXII, CytB and the ribosomal 12S. The study was performed by relative quantification using real time PCR with two reference genes (18S and Cyclophilin). Twenty-seven tumour-non-tumour pairs and 17 additional non-tumour samples were studied.

Although no statistical differences were found between the two groups, the results suggest a down-regulation of the studied genes in tumour samples.

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P0595. RNA alterations in plasma of prostate cancer patients.

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Among molecular markers of prostate carcinogenesis the detection of plasma tumor-specific alterations could be one of the most appealing directions taking into account multifocality and heterogenity of prostate cancer tissue. The detection of circulating, cancer-related RNA molecules in plasma of cancer patients is challeging but potentially effective tool for molecular diagnostic and prognosis.

In this study, we compared the mRNA expression of prostate-associated genes, *PSCA, SFRP1, Akt-1, Her2/neu* to the mRNA expression of *B2M* gene in plasma samples of prostate cancer patients. The RNA isolation was effective in 64% (25/39) plasma samples (volume 800 µl). 4 plasma samples of healthy donors were suggested as control. RNA samples were evaluated using real-time RT-PCR by TaqMan technology. All specimens were positive for *B2M.*

The highest transcript amounts were found for *PSCA*, which relative expression level was dozens as high in 18/21 (86%) samples compared with control. We found insignificant decrease of *Akt-1* and *SFRP1* relative expression levels in 70% and 52% samples accordinally (the others did not express these genes). The presence of *Her2/neu* mRNA in the plasma samples was detected with extremely low concentrations in prostate cancer group as well as in control group.

Our data suggest that plasma *PSCA* mRNA may be a useful tool for detection of prostate cancer although the optimization of RNA isolation is nessecery for increasing of diagnostic sensitivity. This study was support by Applied Biosystems, USA.

P0596. Activating PTPN11 mutations play a minor role in pediatric and adult solid tumors

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The PTPN11 gene encodes SHP-2, a widely expressed cytoplasmic protein tyrosine phosphatase functioning as a signaling transducer. Germ-line PTPN11 mutations cause Noonan syndrome (NS), a developmental disorder characterized by an increased risk of malignancies. Recently, a novel class of activating mutations in PTPN11 has been documented as a somatic event in a heterogeneous group of leukemias. Because of the relatively higher prevalence of certain solid tumors in children with NS and the positive modulatory function of SHP-2 in RAS signaling, a wider role for activating PTPN11 mutations in cancer has been hypothesized. Here, we screened a number of solid tumors, including those documented in NS or in which deregulated RAS signaling occurs at significant frequency, for PTPN11 mutations. No disease-associated mutation was identified in neuroblastoma (N=32), melanoma (N=50), and thyroid (N=85) and colon (N=48) tumors, while a missense mutation previously reported in leukemias was identified in one rhabdomyosarcoma specimen (N=38). Furthermore, a novel missense change, promoting an increase in SHP-2 basal phosphatase activity, was observed in one glioma case. Our data document that deregulated SHP-2 function does not represent a major molecular event in these pediatric and adult tumors, further supporting our previous evidence indicating that the oncogenic role of PTPN11 mutations is cell-context specific.

P0597. Chromosome breakage and apoptotic response to irradiation in unaffected *BRCA1* and *BRCA2* mutation carriers compared with age-matched controls.

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The proteins encoded by *BRCA1*, *BRCA2* and *CHEK2* are known to be involved in cell cycle check points, the repair of DNA breaks and breast cancer susceptibility. However, it is not known whether

individuals who are heterozygous for germ-line *BRCA1* and *BRCA2* mutations have an altered cellular response to irradiation. We have investigated 53 *BRCA1* and *BRCA2* mutation carriers who have never had a malignancy, and age matched unaffected controls.
We found that *BRCA1* and *BRCA2* mutation carriers have normal cell cycle kinetics and apoptotic response to irradiation in peripheral blood lymphocytes compared with controls. However, we detected an increased number of chromosome breaks post irradiation using the G2 assay (P=0.002) and the S phase enrichment assay (P=0.011) in *BRCA1* mutation carriers compared with age-matched controls. *BRCA2* mutation carriers also had an increased number of chromosome breaks per cell compared to their matched controls using the S phase enrichment assay (P=0.045). The repair of other chromosomal aberrations was not statistically different between groups.
Radiation-induced micro-array gene expression data from the peripheral blood lymphocytes of five *BRCA1*, five *BRCA2* mutation carriers and five age-matched controls showed an average reduction in brca1 expression post irradiation in *BRCA1* mutation carriers of 43% (p=0.012), and a non-significant reduction in brca2 protein expression post irradiation in *BRCA2* mutation carriers of 34% compared with controls (p=0.24). We detected a number of consistently altered genes in response to irradiation in mutation carriers with *BRCA1* or *BRCA2* haploinsufficiency and discuss the functional implications of these.

P0598. The canine RAGE - HMGB1 complex, a hand on metastasis?

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Metastasis is one of the major problems when dealing with malignant neoplasias. Accordingly, the finding of molecular targets which can be addressed to reduce tumour metastasising will have significant impact on the development of new therapeutic approaches. Recently, the receptor for advanced glycation end products (RAGE) - high mobility group B1 (HMGB1) protein complex has been shown to have significant influence on invasiveness, growth and motility of tumour cells, which are essential characteristics required for metastatic behaviour. A set of *in vitro* and *in vivo* approaches showed that blocking of this complex resulted in drastic suppression of tumour cell growth.
Due to the similarities of human and canine cancer the dog has joined the common rodent animal model for therapeutic and preclinical studies. However, complete characterisation of the protein complex is a precondition to establish a therapeutic approach based on the blocking of the RAGE-HMGB1 complex in spontaneously occurring tumours in dogs. We characterised the canine *HMGB1* and *RAGE* genes and proteins completely. The canine RAGE gene shows a 2835bp genomic structure, a 1384bp mRNA with a 1215bp protein coding sequence and a 404 amino acid protein. The canine HMGB1 cDNA sequence consists of 2236bp The gene consists of the five exons and four introns of which exon 1 (76bp) and a contig spanning exon 2 - exon 5 (3959bp) were characterised. The deduced protein is a 215 amino acid molecule. Additionally the chromosomal localisations and the gene expression patterns of both genes were characterised.

P0599. *RASSF1A* hypermethylation status in MSI low and MSI high colorectal carcinomas

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RASSF1A tumour suppressor gene is inactivated in a variety of solid tumours, usually by epigenetic silencing of the promoter and/or LOH at 3p. We examined epigenetic and genetic status of the *RASSF1A* gene in 53 colorectal carcinomas (CRCs). 32 CRCs with low MSI and 21 with high MSI were used in the study. 21 high MSI colorectal samples were reselected from a pool of 245 sporadic CRCs evaluated with 5 microsatelite markers (BAT 25, BAT 26, BAT 40, D18S58 and D17S250). DNA was isolated from the tumor and adjacent non - tumour tissue, amplified in polymerase chain reaction (PCR) and analyzed for MSI status. A sample was defined as high-MSI (MSI-H) if more than two of the 5 examined loci showed unequivocal instabilities. Bisulphite treatment of DNA, followed by PCR and direct sequencing of CpG islands revealed the methylation status of the *RASSF1* gene. Hypermethylation of CpG islands was detected in overall 30% of tested colorectal samples, although the extent of methylation varied from a few % to over 70%. No significant differences were found between the frequency of RASSF1A methylation in MSI low and MSI high colorectal carcinomas (P=0.462). Our data demonstrate that RASSF1A is rather frequently inactivated in both, MSI low and MSI high colorectal carcinomas

P0600. Genomic and expression profiling of clear cell renal cell carcinomas with loss of chromosome 3p

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Clear cell renal cell carcinomas (cRCCs) comprise the major subgroup of RCCs. Loss of 3p is the most frequent aberration in this type of tumors and can be found as the sole karyotypic change in about 10% of the cases. The region on 3p that is lost varies from p11.2 to pter, with most of the breakpoints clustering around p14.2. The 3p losses occur by terminal or interstitial deletions as well as by unbalanced translocations of the 3p region with other chromosomes (often chromosome 5). The frequent loss of 3p sequences suggests the presence of one or more tumor suppressor gene(s) that are relevant for (the early stages of) tumor development. Indeed, several candidate genes on chromosome 3p with tumor suppressor activity have been associated with renal tumorigenesis, including the *FHIT* gene on 3p14, the *RASSF1A* gene on 3p21, and the *VHL* gene on 3p25. In this study, we performed molecular fine-mapping of the genomic regions on chromosome 3 involved in RCC initiation and progression. We have selected a set of 36 clear cell renal tumors with 3p aberrations, and with either a relatively good or poor outcome of the disease. Using high-resolution array CGH we were able to reveal the most commonly deleted regions on 3p. By integrating the genomic profiles with expression profiles of these tumor samples, we aim to identify the genes on 3p of which the expression is affected by copy number loss and which, thus, are candidate genes involved in clear cell renal tumorigenesis.

P0601. Microsatellite analysis in sporadic renal cell carcinoma (RCC) and its prognostic value

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Purpose: Sporadic renal cell carcinoma (RCC) is one of the most common urological malignities in adults (85%). The prognosis of RCC is very poor because of high mortality and unpredictable progression after tumor abstraction. We correlated allelic loss on chromosome 3p with clinicopathologic data of patients with conventional renal cell carcinoma.
Material and Methods: Our RCC sample collection was obtained after radical nephrectomy from 60 patients. DNA was isolated from tumor cells, nontumorous renal cells and blood. Microsatellite analysis of the D3S1289 and D3S1560 was performed by fragmentation analysis on GA ABI PRISM 3100-Avant.
Results: Microsatellite analysis showed loss of heterozygosity (LOH) on chromosome 3p in 71,7 % of the RCCs. We found a correlation between LOH and nuclear grade (p=0.08) on the cut-off of statistic significance, but no correlation with other parameters.
Conclusions: LOH on loci of the D3S1289 and D3S1560 may provide prognostic significance in patients with the RCC.
Research is supported by MSM 0021620808.

P0602. Genetic differences of serous Fallopian Tube Carcinoma, Serous Ovarian Carcinoma and an aggressive borderline malignancy of the ovary: a Genome-Wide Array-Based Comparative Genomic Hybridization Study

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The molecular relationship between serous ovarian carcinoma (OVCA) and serous Fallopian tube carcinoma (FTC) is still unclear. We have performed Array-Comparative Genomic Hybridization studies, analysing 14 representative FTC and 14 matched OVCA cases at a genome-wide scale with a medium resolution. In addition, we have compared the results with data of a clinically agressive serous borderline malignancy of the ovary obtained with exactly the same platform. The studies indicate that the analysed OVCAs and FTCs displayed both common and more distinctive patterns of changes. In contrast, the investigated borderline case did not show a similar pattern of changes. Zooming in on the genes present in the altered regions results in the identification of already known genes implicated in gynaecological malignancies, such as cMyc, ERBB2, CCNE1 and Akt2. New genes found include Brg1, whose product has been implicated in a BRCA1-linked pathway, suggesting that this pathway may also play a role in sporadic cancers. A further development of DNA-tests based on results of array-CGH might be useful for diagnostic purposes, especially to discriminate between borderline tumors and OVCAs and FTCs and be instrumental to prevent erroneous chemotherapies for borderline patients.

P0603. CD24 as a unique immunphenotyping marker for Chronic Myeloid Leukemia-Stem Cell

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The purpose of this study was to find a marker that diminishes chronic myeloid leukemia-stem cells (CML-SCs) from normal stem cells in a mouse model system of CML. As a mouse model retrovirus transduction model was employed using a vector expressing p210/BCR/ABL and the green fluorescent protein (GFP). Transplantation studies of BCR/ABL infected cells in recipient mice demonstrated an aggressive myeloproliferative disease within ~3 weeks, as has previously been observed with this approach. Phenotypic analysis of bone marrow from BCR/ABL mice showed contribution by the transduced cells to multiple lineages, suggesting a primitive stem/progenitor cell gives rise to the disease. Subsequent analysis of primary cells that arise *in vivo* showed the leukemia-initiating activity resides in cells that are Sca-1+/c-kit+/Lin-. Further, comparison of the BCR/ABL mice and GFP control mice revealed preferential over-expression of CD24 (heat-stable antigen) in the primitive leukemic cells (~90%), demonstrating a possible BCR/ABL induced activation of the antigen. Sorting and serial transplantation studies of CD24+ vs. CD24- cells from BCR/ABL mice showed that the leukemia-initiating activity resides in the $CD24^{+}$/ Lin^{-} population. Collectively, these data indicate that BCR/ABL expression increases proliferative of primitive CML cells and up-regulates the CD24 antigen. We suggest that the CD24 antigen represents a potential marker to distinguish normal from CML stem cells and that it may confer specific functional properties on the CML population.

P0604. NFY drives basal transcription of the human *TLX3*, a gene overexpressed in T-acute lymphocytic leukaemia

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Based on a knocked-out mouse model and a few expression studies, TLX3 is regarded as a homeobox gene crucial for the development of the autonomic nervous system. Despite an involvement in normal ematopoiesis has been excluded, its expression can be detected in 20% of children and 13% of adults T-cell acute lymphocytic leukaemia (T-ALL) samples, frequently in association with chromosomal rearrangments involving 5q35, where the gene is located.
We have focused on the identification of elements and factors playing a role in the TLX3 physiological expression regulation, and therefore likely to be involved in cancer development. In particular, after identifying the transcription start points, we have made use of in vitro tranfection assays to demonstrate that the 5'-untranslated region of the gene is necessary for the basal promoter activity in cell lines from different origin. By site-directed mutagenesis, two tandem CCAAT boxes have been localized as critical elements of this region. In vivo chromatin immunoprecipitation and electrophoretic mobility shift assays have indicated that nuclear factor Y (NFY) recognizes these sites in all the analyzed cell lines. The physiological role of such an interaction has been confirmed by means of a dominant negative version of the NFY transcription factor, that has turned out to decrease both in vitro TLX3 promoter activity and endogenous amount of mRNA. We propose that proper cell specific regulation of the human TLX3 gene requires the interaction of the NFY-proximal sequence with long-range tissue-specific cis-elements which we are searching at present.

P0605. A preliminary study: Evaluation of Manisa propolis effect on leukemia cells obtained from leukemia patients with telomerase activity

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Propolis is a resinous material collected by the honeybees and obtained from beehives. Recently the characteristics of this product have attracted great attention in terms of anticancer, immunostimulatory, antiviral and antibacterial effects. Flavanoids and caffeic acid phenyl esters in propolis have been shown to be antimutagenic and anticarcinogenic. In addition there have been a considerable number of studies investigating the underlying mechanisms of those effects of propolis. We have previously shown that propolis inhibited the expression of telomerase by decreasing the hTERT level in T-cell acute lymphoblastic leukemia cell line. The aim of this study is to investigate the effect of propolis prepared in different concentrations on hTERT in the leukemia cells obtained from leukemia patients.
Telomerase activity in the cell cultures of bone marrow samples in 4 cases with leukemia have been evaluated following propolis treatment in 3 different concentrations after 24 and 48. Cell viability of propolis-treated and control cultures were analyzed by XTT assay. The LightCycler instrument was used for the quantification of hTERT.
Propolis treatment was not effective in juvenile myelomonocytic leukemia cells, however, significantly both positive and negative differences were observed in the hTERT of pre-B-cell leukemia. Particularly, propolis decreased the hTERT significantly in the case with high leucocyte count.
Propolis may be effective in a positive or negative way on hTERT in the pre-B leukemia. Determining the way propolis has an effect on those cells needs to be evaluated by analyzing the individually varying parameters in leukemia with the hTERT in further studies.

P0606. Telomerase activity in pediatric and adult-onset hematological malignancies

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Telomerase is a reverse transcriptase enzyme which synthesizes telomeric DNA (TTAGGG)n in dividing cells. It is highly activated in germ-line and in immortal cells. The level of telomerase activity may be correlated to the diagnosis, prognosis and possible therapeutic approaches to the hematological malignancies. Its activity decreases with age in the absence of a malignant process. In this study we measured the level of telomerase activity in different pediatric and adult-onset hematological malignancies to make comparisons between those diseases investigated and to evaluate whether there is any age dependendency or not. Those ratios were also compared to the prognostic factors described previously. The online real-time reverse-transcriptase PCR was used for the quantification of hTERT in peripheral blood (PB) and bone marrow (BM) in 32 cases with acute lymphoblastic leukemia (ALL), 13 cases with acute myeloblastic leukemia (AML), 10 cases with myelodysplastic syndrome (MDS), 7 cases with multiple myeloma (MM). PB hTERT correlated significantly with BM levels in acute leukemia and in MDS. Highest hTERT were observed in ALL patients followed by MDS, AML and MM patients respectively. The average relative-ratios of BM and PB hTERT were 175.30±206.55 and 99.03±152.27 in ALL, 9.28±12.47 and 8.61±13.32 in AML, 34.23±39.52 and 22.35±24.67 in MDS. The average BM hTERT was 4.82±4.91 in MM patients. No correlation was observed

between the telomerase activities and the age in the hematological malignancies investigated. The known prognostic factors were not shown to have significantly correlation to the telomerase activity in all malignancy groups.

P0607. Expression of a new cancer/testis gene ,TSGA10, in patients with acute lymphoblastic leukemia (ALL).

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Among tumor antigens identified to date Cancer/testis (C/T) antigens, are a group of highly attractive targets for immunotherapy and cancer vaccines. To date more than 50 C/T have been identified. Testis-Specific Gene A10 (TSGA10) is a recently identified C/T gene which is expressed normaly in testis, fetus, and frequently in human cancers. It can make the gene a candidate for immunotherapy and for detection of minimal residual disease (MRD). We previously demonstrated TSGA10 expression during spermatogenesis. There is also evidence for potential TSGA10 involvement in cell proliferation and/or differentiation. Antibody against TSGA10 antigene has been observed in some cancers.
Here we demonstrated expression of TSGA10 by semi-quantitative RT-PCR in 44 (84.6%) out of 52 bone marrow samples and all peripheral blood samples from patients with acute lymphoblastic leukemia (ALL), while there is no expression of TSGA10 in normal blood samples.
Presence of TSGA10 expression in ALL may open a window to functional study of mitotic checkpoint proteins in leukemia. RT-PCR of TSGA10 may help in detection of residual clonal cells leading to early diagnosis and better prognostic qualification of the disease.

P0608. Detection of monosomy 3 in uveal melanoma: A comparison between interphase fluorescence in situ hybridization on cultured cells and on nuclei isolated from paraffin-embedded tissue

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Uveal melanoma is the commonest malignancy of the eye among adults with an annual incidence rate between 0.49 and 0.76 per 100.000 in the Caucasian population. Up to 53% of the patients die of metastatic disease. It is important to identify high risk patients, so that such individuals could be monitored for the presence of liver metastases and could be offered adjuvant chemotherapy or liver resection surgery at an early stage. Unfavourable prognostic factors among others are tumor size, ciliary body involvement, the presence of epithelioid cells and specific extravascular matrix patterns. Monosomy of chromosome 3 is also a significant indicator of poor prognosis in uveal melanoma. The identification of this monosomy in tumor tissue is therefore very important. The aim of this study was to compare the detection rate of monosomy 3 between cytogenetic analysis and interphase fluorescence in situ hybridization (FISH) on cultured cells and interphase FISH on nuclei isolated from paraffin-embedded tissue. Both techniques were performed in a series of 50 enucleated uveal melanomas. FISH on nuclei isolated from paraffin-embedded tissue showed monosomy 3 in 62% of the cases while combining karyotyping and FISH on cultured cells a monosomy of chromosome 3 was detected in only 38% of the cases. We can conclude that the use of interphase FISH on nuclei isolated from paraffin-embedded tissue increases the possibility to identify patients at high risk for metastases.

P0609. Rapid Quantitative Detection of WT1 Expression Level by Real-Time RT-PCR for Evaluation of Minimal Residual Disease (MRD) in Pediatric Acute Lymphoblastic Leukemia

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The submicroscopic levels of leukemic cells (MRD) in peripheral blood samples from children with acute lymphoblastic leukemia (ALL) have clinical importance. We aimed to develop a quantitative method based on the real-time quantitative RT-PCR (RQ-PCR) in order to monitor WT1 level in children with ALL and assess its prognostic importance. Furthermore, we wished to compare the sensitivity, efficiency, and reliability of this method with the conventional semi-quantitative RT-PCR (CQ PCR).
Fourteen newly diagnosed ALL children were included in this study. The peripheral blood samples were collected before induction, at the second week of induction, at the start of consolidation and beginning of the maintenance phase of chemotherapy. The last blood sample was collected from each patient at random 2 to 6 month after the maintenance phase started. For Real-Time RT-PCR we have used LightCycler device, SYBR Green I dye and WT1 primers with 90bp PCR products.
The proportion of patients with detectable WT1 was 92% at diagnosis and 42% at the second week of therapy, 38% at the start of consolidation, and 0% at the beginning of maintenance. WT1 could not be detected in any of the last samples. The absence of WT1 showed accordance with the clinical course of patients. CQ PCR and RQ PCR results were correlated together.
These preliminary results indicate that real time PCR can be employed to monitor MRD in the leukemic patients during chemotherapy. Although the CQ PCR results were strongly correlated with RQ PCR but it had lower sensitivity and reliability.

P0610. BAC Array CGH analysis of primary childhood acute lymphoblastic leukaemias and their recurrencies

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Array CGH analysis has been applied to DNA of childhood acute lymphoblastic leukemia samples (n=28) for the detection of copy number changes. The samples represent three different disease stages (first occurrence, first and second relapse) of 10 patients. The Array CGH chip used is a custom made BAC Array a resolution of at least 1 Mb or less.
In total, 230 copy number gains versus 214 copy number losses were found. The mean number of aberrations was five gains and six losses per sample. In detail, aberration count ranged from no aberration to 36 copy number gains or up to 22 deletions per sample. The most aberrations were detected within the group of *TEL/AML1* negative tumors. The mean count of copy number changes per sample was 18, composed of eight losses and ten gains per sample. Here, loss of chromosome 9 material and copy number gains of chromosome 21 were found in nine, and in eight of 18 samples, respectively. In contrast, the four *TEL/AML1*-fusion gene positive patients showed the least number of copy number changes. In detail, it ranged from none to nine copy number gains, and from two up to 13 deletions per sample. The chromosome bands 12p13-p12 encompassing the *TEL*-gene was lost most frequently. This gene is represented by two BACs on the Array chip.
FISH analysis will be performed to confirm these results as well as other copy number changes with a resolution of less than 1 Mb.

Po05. Molecular and biochemical basis of disease

P0611. The implication of DNA damage in neurodegenerative conditions

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DNA damage has been implicated in numerous neurodegenerative conditions. We showed that P38 MAPK is up regulated / phosphorylated and participate in death signaling following DNA damage. In this experimental model, primary embryonic cortical neurons were prepared from E14-16 rat embryos. A density of 1.2×10^5 cells/well plated on the wells, pre-coated with poly-D-lysine. Camptothecin, the topisomerase-1 inhibitor, (10^{-5} M) was added to neuronal culture after 24 hours. After 4,6,24 and 48 hours , expression of the P38 and ATF-

2 was studied using primary antibody in the Immunocytochemistry technique, and number of healthy and death nuclei were counted by cell lysis buffer. Then percentage of the healthy , death and expression of the cells was analyzed by one way ANOVA followed by Tukey's post test . Percentage of the expression of P38 was 4%, 20%, 40% and 55%, and percentage of the survival was 95%, 85%, 64% and 50% for 4, 6, 24 and 48 hours, respectively. The expression of ATF-2 was also 3%, 20%, 30%, 45% and percentage of the survival was 97%, 85%, 64% and 50%, respectively. Percentage of the expression and survival of the P38 neurons for 24 hours were 40 and 64 percent and it was for ATF-2 , 30 and 64 percent respectively which these results compared to control were increased significantly ($p<0.05$).Expression of the proteins at 4 hours was not changed significantly. Expression of the P38 and ATF-2 was increased simultaneously. Thus, in this model, Camptothecin induces neuronal death by stimulation P38-ATF-2 pathway.

P0612. Mutation screening of the APC gene in 134 Spanish Families.

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Background: Familial adenomatous polyposis (FAP) is an inherited, autosomal dominant syndrome caused by germ-line mutations of the adenomatous polyposis coli (APC) gene. Affected individuals develop hundreds to thousands of colonic polyps. More than 700 mutations have been described until now along the gene and it was found that the same APC mutation could cause different clinical manifestations. However, it has been suggested the relationship between the localization of the mutation and specific phenotypes associated with the disease.

Matherial and Methods: We study 134 unrelated patients with diagnosis of FAP and their relatives. Genomic DNA were obtained from affected individuals after informed consent and mutation analysis of the whole coding sequence of APC gene was done by dHPLC .The results obtained were confirmed by DNA sequencing.

Results: Sequence changes of the APC gene have been detected in 45% of the affected patients We have identified twenty novel mutations: three missense mutations, nine small deletions and two indels that create a new stop codon and produce a truncated protein, and six nonsense mutations. Nineteen mutations corresponded to exon 15 and one to exon 13. Four novel silent polymorphisms were founded in exon 15 and 13 of the gene.

Conclusion: The study of mutation status in the affected members as well as in the healthy individuals of each family has confirm that mutations are causative. We also describe the patient's phenotypes associated with these mutations. The description of every new mutation finding may contribute to a better knowledge of these severe disease.

P0613. CTG Repeat Polymorphism at the Myotonic Dystrophy Locus in Healthy Iranian Population

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Myotonic Dystrophy type1 is caused by large and unstable expansion of CTG repeats in DMPK gene which is located on 19q13.3 . According to the hypothesis that expanded (CTG)n alleles originated from large size normal alleles, there is a correlation between the prevalence of DM1 and the frequency of large size normal alleles in a population . So DM1 is considered to be more prevalent in Europe and Japan but it is rare in Africa. It has also been proposed that DM1 is not prevalent in other parts of Asia including Iran.

To determine the distribution of alleles in healthy Iranian population and the frequency of large size normal alleles.

Two hundred healthy individuals from different ethnic groups who live in Iran participated in this study. A polymerase chain reaction was conducted to determine the size of the alleles.

So far, our data reveals that 23.70% of alleles had 5 repeats, 23.25% had 6-8 repeats, 45.75% had 9-17 repeats and 7.25% of alleles had CTG repeats of more than 18. The most repeat number of CTG among normal allele was 28.

The frequency of CTG≥18 is 9.83% in Western Europe and 9% in Japan which suggests that there is a correlation between the prevalence of DM1 in a population and the frequency of alleles with CTG≥18. What we have concluded up to now from our data shows that the frequency of alleles with CTG>18 is comparative to Western Europe and Japan.

P0614. Analysis of polymorphisms metabolisms genes (CYP19, GSTM1, GSTT1 and NAT2) in endometriosis patients with different efficiency of hormone-modulate therapy.

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Endometriosis is an estrogen-dependent disease of women of reproductive age. The main direction of conservative therapy of endometriosis patients is the recovery of unstable hormone status with anti-estrogens drugs. However, some patients are resistant for hormone-modulate therapy and have severe side effects. Retrospective analysis of the results of hormone therapy allowed to divide patients to two groups according to hormone therapy efficiency. Group 1(54 patients) revealed positive responses to combined surgical and therapeutic treatment. Some women have demonstrated conspicuous resistance to this kind of treatment and were arbitrarily attributed to group 2 (63 patients). An increased frequency of A1 allele of the CYP19 gene was observed in group 2 as compared to group 1 (32% versus 17%, $p<0.01$). Carriers of the A1/A6 genotype were registered in 30.6% of group 2 as compared to 4.7% in group 1 ($p<0.01$). Analysis of combination of functionally impaired alleles of the GSTM1, GSTT1 and NAT2 revealed significant difference between two groups of patients. The combination of the GSTT 0/0 and NAT2 S/S ($p<0.05$; OR 8.3), GSTM1 0/0 and NAT2 S/S ($p<0.01$; OR 8.6), GSTM1 0/0 and GSTT1 0/0 ($p<0.05$; OR 10.5) genotypes significantly increased in group 2. Total analysis of the GSTM1, GSTT1 and NAT2 genes revealed significantly higher frequency of functionally impaired genotypes in group 2 as compared to group 1 (68.6% and 13.0% respectively, $p<0.001$,OR 14.6). These data suggest that the polymorphisms metabolisms genes is associated with different effect of hormone therapy in endometriosis patients.

P0615. Mutation in gene encoding RANK in Iranian familial expansile osteolysis pedigree

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Familial expansile osteolysis (FEO) is a rare bone dysplasia which is transmitted as an autosomal dominant trait. Generalized features are altered trabecular pattern or modeling abnormalities. Hearing loss is an early manifestation during childhood and cervical resorbtion of teeth is common. Osteolytic lesions usually develop in the long bones during early adulthood. The disease was linked to chromosome 18 in a large afflicted Irish pedigree in 1994, and the associated gene, TNFRSF11A, encoding receptor activator of nuclear factor-κ B (RANK) was discovered in 2000. RANK is highly expressed in bones and is essential in osteobalst formation. The Irish pedigree and three subsequently identified pedigrees form America, Germany and Spain, all shared a common 18 base pair insertional mutation in the gene. There is evidence that the mutation prevents appropriate trafficing of the protein to the cell surface and also increased constituitive activation of RANK. We now report the same mutation in an Iranian FEO pedigree, which is the first reported FEO pedigree of non-European descent. The mutation was identified by sequencing and an intragenic haplotype for the mutated allele is presented.

P0616. Novel mutation in *SLC5A2* encoding SGLT2 in a Portuguese patient with autosomal recessive renal glucosuria

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Renal glucosuria (RG) (OMIM #233100), an autosomal-recessive disorder characterized by renal glucose wasting in the absence of hyperglycaemia or other proximal tubular dysfunction, results from mutations in *SLC5A2* gene encoding the sodium/glucose co-transporter type 2 (SGLT2). In this work, we report a novel mutation Val296Leu (886G>C) in a non-consanguineous Portuguese family with autosomal-recessive RG. The index case is a 15 year-old patient, asymptomatic but with glucosuria and with normal glucose tolerance. Sequence analysis of *SLC5A2* genomic DNA revealed a relatively common SGLT2 IVS7+5 G>A mutation (a donor splice site mutation found to be associated with some residual glucose re-absorption and comparably low glucose excretion) transmitted from the patient´s father and a novel mutation Val296Leu (886G>C) involving a highly conserved residue. The mother was heterozygous for this last mutation. These findings confirm that mutations in the *SLC5A2* gene are responsible for recessive renal glucosuria.

P0617. Microarray based mutation analysis of ABCA4 gene in several retinal dystrophies from Spain

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Genetic variation in the ABCA4 (ABCR) gene has been associated with several distinct retinal phenotypes, including Stargardt disease/ fundus flavimaculatus (STGD/FFM), cone-rod dystrophy (CRD), retinitis pigmentosa (RP) and age-related macular degeneration. The current model of genotype/phenotype association suggests that patients harbouring deleterious mutations in both ABCR alleles would develop RP-like retinal pathology. Here we describe the spectrum of mutations in ABCA4 gene founded in several phenotypes associated in Spanish population.

Of 61 families recruited, 7 were diagnosed as Autosomal Dominant Macular Dystrophy, 26 were cone-rod dystrophy and 28 were classified as typical RP.

For ADMD families three mutated alleles were detected in three families in heterozygous state (42%). For family 59 a complex allele G1961E+S2255I was detected but it seems that the mutation no cosegregate in this family. For family 92 a mutation in heterozygous state, I156V, was detected. And a mild allele (R943Q) was detected for family 105.

For CRD families, for 11 of them a mutation were detected, for five families two mutations were found and for three families only one mutated allele were detected. So we could detect almost one mutated allele in eleven families out of 26, represented 42%. For 28 classical RP families, no mutations were founded for 18 families and only in the remaining 10 families one mutated allele was detected. So we detected almost one mutated allele in the 35% of the RP cases.

P0618. Polymorphisms in the ACE gene are associated with recurrent spontaneous miscarriages and placental insufficiency

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The pathogenesis of recurrent spontaneous miscarriages (RSM) and placental insufficiency (PLI) is rather complex and presumably involves interaction of several genetic and environmental factors. The renin-angiotensin system (RAS) contributes to the pathogenesis of several diseases including fibrosis in the heart, kidney, lung, placenta and thus most probably might be involved in RSM and PS. The key enzyme in RAS is the ACE which converts angiotensin I to the potent vasoconstrictor angiotensin II.

The I/D polymorphism of the ACE gene was investigated by PCR in placentas from 41 childbirth women of without RSM and without PLI in anamnesis (control group I), and in 49 placentas from childbirth women without RSM and with clinically and morphologically proved PLI (II group). Group III included 23 placentas from patients with RSM and without PLI, and IV group included 24 placentas from patients without RSM and with PLI.

ACE genotype frequencies D\D D\I and I\I were significant difference ($p<0,01$: df6) in 4 groups studied (Table.I).

Table I. ACE gene genotypes frequencies in 4groups studied

genotype	Igroup(n=36)	IIgroup(n=54)	IIIgroup(n=23)	IVgroup(n=24)
D\D	22,2%	46,4%	47,8%	16,6%
I\D	58,3%	29,6%	43,5%	7,5%
I\I	19,5%	24%	8,7%	45,9%

ACE gene polymorphism is partly involved in the origin of RSM and PLI. ACE genotype could be treated as a disease modifier factor rather than as a disease susceptibility contributor.

P0619. Molecular pathology of two special cases of acute intermittent porphyria

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Porphyrias are mostly inherited disorders caused by decreased activities of the enzymes in the heme biosynthetic pathway. Heme is synthesized by an orchestrated cascade of eight enzymes. Defects in seven of these enzymes cause porphyrias, each of them characterized by a typical spectrum of accumulated and excreted porphyrins and their precursors. Four of them are described as acute hepatic porphyrias (AP), which share possible precipitation of acute attacks. Acute intermittent porphyria (AIP), caused by porphobilinogen deaminase deficiency (PBGD), is the most frequent among hepatic porphyrias.

During systematic genetic analysis of AIP patiens, diagnosed in Czech and Slovak Republics, we found three novel mutations and five previously described mutations in seven unrelated patients. Two of these patients are the subject of this presentation: In a 15-year-old boy with abdominal and subsequent neurological symptomatology, 966insA (led to stop codon after 36 completely different amino acids compared to wt-sequence) mutation within PBGD was found (*de novo* mutation). In another patient (female with acute attack in personal history), two mutations [518G>A (R173Q), and 610C>A (Q204E)] were found to be located in the same allele of exon 10. Structural modeling and the expression studies were performed.

The probability of a life-threatening porphyric attack in AIP is a significant personal burden and creates a challenge for counseling and medical management. Introducing molecular biology techniques to the diagnosis and heme arginate to the treatment of acute attacks increase our chances for effective treatment of AIP.

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P0620. Molecular characterization of the first missense mutation in the fibrinogen Aalpha-chain gene causing afibrinogenemia.

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Congenital afibrinogenemia (MIM#202400) is a rare coagulation disorder characterized by very low or unmeasurable levels of functional and immunoreactive fibrinogen in plasma, associated with a hemorrhagic phenotype of variable severity. It is transmitted as an autosomal recessive trait (prevalence 1:1.000.000) and is invariantly associated with mutations affecting one of the three fibrinogen genes (*FGA*, *FGB* and *FGG*, coding for Aα, Bβ and γ chain, respectively). Fibrinogen is secreted by hepatocytes as a hexamer composed of two copies of each chain; the lack of one chain has been demonstrated to prevent its secretion. Most genetic defects causing afibrinogenemia are truncating mutations, whereas only few missense mutations (4) have been identified so far, all located in *FGB*.

In this study, direct sequencing of the fibrinogen genes in a 32-years-old afibrinogenemic Italian male identified the first missense mutation

(Met51Arg) located in the Aα-chain gene leading to a quantitative fibrinogen deficiency. The patient was a compound heterozygote for a previously described frameshift mutation (1215delT) in the same gene. Met51Arg involves a residue located at the very beginning of the coiled-coil domain, in a region previously demonstrated to play a pivotal role in hexamer formation. *In-vitro* expression experiments in COS-1 cells showed that Met51Arg abolishes secretion of the hexameric molecule, even though traces of not completely assembled trimeric intermediate were found in conditioned media. Western blot analysis performed on the proband's plasma confirmed the presence in vivo of the trimeric fibrinogen, further supporting the hypothesis that the mutation alters the final steps of fibrinogen assembly.

P0621. Dysferlin interacts with AHNAK: secondary reduction of AHNAK in dysferlinopathy patients

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Mutations in dysferlin cause Limb Girdle Muscular Dystrophy (LGMD) 2B, Miyoshi myopathy (MM) and distal anterior compartment myopathy. Dysferlin is primarily localized at the sarcolemma in skeletal muscle and has been implicated in membrane repair processes. Its homologue, myoferlin localizes to the sarcolemma and the nuclear membrane and is highly expressed during development. To further define the dysferlin protein complex in skeletal muscle and to obtain functional insight in dysferlin, we selected two single-domain antibody reagents (VHH) that recognize different epitopes of dysferlin.

Co-immunoprecipitation coupled to MALDI allowed the identification of proteins that interact with dysferlin. Amongst these was AHNAK, previously implicated in cortical actin cytoskeleton organization and cell membrane cytoarchitecture. GST-pull down and co-IP studies confirmed the direct interaction between AHNAK and dysferlin or myoferlin. Moreover, immunofluorescence microscopy revealed a secondary reduction of AHNAK at the sarcolemma in dysferlinopathy patients. As AHNAK does not contain transmembrane domains, we propose that dysferlin and myoferlin anchor AHNAKs to the membrane, which provides a link between dysferlin and actin filaments.

P0622. Characterization of the intracellular location of AID isoforms

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A B-cell specific molecule, activation-induced cytidine deaminase (AID), has been recently shown to be essential and sufficient for B-cell affinity maturation processes such as somatic hypermutation (SHM) and class-switch recombination (CSR). This protein is also involved in gene conversion, which occur in the chicken.

The functional model proposed for the AID protein suggests that it generates mismatch of DNA base pairs directly through DNA edition or indirectly through RNA edition.

We have analyzed by RT-PCR AID expression in B and non-B cell lines and characterized five differents isoforms: complete AID, AIDdel(ex4)(30bp) lacking 30bp from 5' end of exon 4, AIDdel(ex4) lacking exon 4, AIDins(IVS3) including intron 3 and AIDdel(ex3,4), a novel isoform lacking exons 3 and 4 (GenBank accession numbers: NM020661, AY536516, AY536517, AY541058, AY534975). All isoforms maintain the N-terminal region and differ at the carboxi-termini. We next created constructs containing each isoform with a FLAG-epitope and we transfected these constructs into COS7 cells. Inmunofluorescence analysis showed that all isoforms located at the cytoplasm. However, in 20% of transfected cells, isoforms lacking exon 4 were present also in the nucleoli. Thus, our results indicate that AID isoforms may have different intracellular location and suggest that they may exhibit different function.

P0623. From childhood to adulthood: phenotypic variability in neurodegenerative disorders due to GFAP mutations

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Alexander disease is related to mutations in the *GFAP* gene encoding the glial fibrillary acidic protein (GFAP), a major intermediate filament of mature astrocytes. Identification of the molecular defect allowed to describe the phenotypic spectrum of the disease, first reported in early childhood.

We identified mutations in *GFAP* gene in 16 patients. In 13 patients onset of the disease occurred before 2 years: all of them had a suggestive MRI, 9 a progressive megalencephaly and 7 at least one episode of seizures. Only 2 had a classical infantile course with regression before 2 years while others patients displayed non specific mental retardation before onset of seizures or motor impairment. In 2 patients disease begun in adulthood with palatal myoclonus. Precocious puberty and paroxystic dysphagia were the first symptoms in one patient affected with juvenile-onset disease. Surprisingly MRI revealed a tumoral chiasmatic lesion in an infantile Alexander patient. The association of Alexander disease and glioma, two primitive astrocytic diseases, seems not to be fortuitous and has been discussed in literature.

In conclusion, Alexander disease could be revealed by classical infantile onset but also by atypical symptoms like non specific mental retardation, brainstem dysfunction, spinocerebellar atrophy or anorexia, precocious puberty, tumor-like lesion on MRI.

P0624. A functional study of a novel *GFAP* complex allele detected in a familial adult form of Alexander disease

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Alexander disease is a rare neurological genetic disorder characterized by progressive white-matter degeneration associated with presence in astrocytes of cytoplasmic aggregates, called Rosenthal fibers, including the intermediate filament glial acid fibrillary protein (GFAP). Infantile, juvenile and adult forms of the disease have been defined on the basis of age of onset and most of them have resulted to be caused by heterozygous *GFAP* mutations.

We have analyzed the *GFAP* gene in 12 Italian families, two of them with more than one affected over two generations, identifying ten different alleles carrying 11 missense mutations. Interestingly, two of these, lying coupled on the same allele, were detected in the affected members of a family characterized by recurrence of an adult onset of Alexander disease.

To investigate the functional effect of this latter complex allele and a possible cumulative effect of the two mutations, we have generated expression constructs encoding the wild type GFAP and its mutant forms (namely the double mutations, the two single mutations and a mutation, R239C, already known to display deleterious effects) in frame with the Green Fluorescent Protein coding sequence. After transfection in astrocytoma U251 cells, fluorescence microscope analysis revealed that the construct carrying the two coupled *GFAP* mutations produced patterns of GFAP aggregates characterized by intermediate severity between the wild type and R239C GFAP proteins. Our results are therefore consistent with a phenotype-genotype correlation between the severity of the disease and the *in vitro* amount of *GFAP* mutation dependent formation of cytoplasmic aggregates.

P0625. Investigation of the HLA-DRB1 locus in alopecia areata

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To further evaluate the nature of the HLA association with alopecia areata (AA), we investigated the HLA-DRB1 locus in 161 AA patients and 165 matched controls from Belgium and Germany. HLA-DRB1 typing was performed by a recently established method that employs a combination of PCR-SSP (sequence specific priming) and Pyrosequencing™ technology.

No significant differences were observed for HLA allele groups DRB1 *01, *15, *16, *11, *13, *14, *07, *09, and *10. HLA-DRB1*03 was found to confer a protective effect (7.5% versus 13.6%, p=0.011). Additional genotyping at the allelic level revealed a significant difference of HLA-DRB1*0301 between patients and controls (6.8% versus 11.2%, p=0.048). The DRB1*04 allele group was confirmed as a risk factor for the development of AA (20.8% versus 13.3%, p=0.012) with the allele DRB1*0401 accounting for the greatest proportion of the effect (13.4% versus 7.3%, p=0.014). Results obtained after subgrouping of the patients according to age at onset, severity and family history of the disease suggests that the genetic effects of the HLA system are strongest in familial cases of the disease.

P0626. The detection of large rearrangements on 16p13.3 causing ATR-16 by Multiplex Ligation-dependent Probe Amplification.

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Genomic deletions involving the α-globin gene cluster on chromosome 16p13.3 are the most common molecular cause of α-thalassemia (approx. 80-90% of cases) and Alpha-thalassemia Mental Retardation Syndrome (ATR-16). At present the molecular tests commonly used to identify deletion types of α-thalassaemia or ATR-16 are gap-PCR, Southern blot or Fluorescent In Situ Hybridization (FISH) analysis. However, the applicability of these techniques is limited to known deletions, may involve radio-activity, is dependent upon the hybridization probes available and may require time consuming and laborious cell culture to generate metaphase chromosome spreads. We have developed a rapid and simple technique based on Multiplex Ligation-Dependent Probe Amplification for high resolution mapping of rearrangements involving the tip of the short arm of chromosome 16. We identified three deletions causing ATR-16 in Dutch patients suffering from mild to moderate mental retardation. Because of its robustness and simplicity, this technique should become the standard for the detection of (large) rearrangements causing hemoglobinopathies, ATR-16 and other diseases, in laboratories capable of performing automated DNA fragment analysis.

P0627. Identification of mutations in X-linked Alport Syndrome by Temperature Gradient Capillary Electrophoresis using the 96-capillary SpectruMedix platform

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X-linked Alport syndrome is a hereditary neuropathy associated with haematuria, progressive renal failure, sensori-neural deafness and characteristic eye signs. It is caused by mutations in the COL4A5 gene and

approximately 65% of mutations reported are point mutations. Small insertions and deletions account for 20% of mutations with gross rearrangements also described.

We have developed a mutation scanning approach for Alport syndrome by amplifying all 51 exons in 24 non-flourescent multiplex PCR assays. TGCE is performed on the SpectruMedix machine, a high- throughput 96-capillary platform which uses ethidium bromide staining to identify heteroduplexes.

The COL4A5 gene has GC rich fragments and can be problematic for heteroduplex analysis but the TGCE technology allows heteroduplexes to be identified using one temperature range. The data is analysed using Revelation software which is specifically designed for the identification of heteroduplexes. This enables traces to be overlaid and shifts identified.

Heteroduplex peak shifts are characterised by sequence analysis to identify pathogenic mutations and to eliminate changes caused by polymorphisms. The COL4A5 gene is well suited to this approach as it has a low number of polymorphisms. Using this approach mutations have been identified in 14/48 patients, 5/14 males and 9/34 females.

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P0628. Analysis of mitochondrial haplogroups in Persian patients with Alzheimer's disease

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Alzheimer's disease (AD) is the most common form of dementia in the elderly in which interplay between genes and the environment are supposed to be involved. Mitochondrial DNA (mtDNA) has the only non-coding regions at the displacement loop (D-loop) region that contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. mtDNA has already been fully sequenced and many subsequent publications have showed polymorphic sites, haplogroups and haplotypes. Haplogroups could have important implications to understand association between mutability of the mitochondrial genome and disease. To assess relationship between mtDNA haplogroup and AD, we sequenced the mtDNA HVS-I in 30 AD patients and 100 control subjects. We have found that haplogroups H and U are significantly more abundant in AD patients (P= 0.016 for haplogroup H and P=0.0003 for haplogroup U). Thus, these two haplogroups might act synergistically to increase the penetrance of AD disease.

P0629. Investigation of polymorphisms and common deletion in coding region of human Mitochondrial DNA in 20 Persian patients with Alzheimer's disease (AD)

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Alzheimer's disease (AD) is the most common form of dementia in the elderly in which interplay between genes and the environment are supposed to be involved There are several disparate lines of evidence pointing to mitochondrial involvement in AD. Somatic mtDNA rearrangement mutations have been observed to be increased in AD brains. Also, certain germ line mtDNA mutations have been also associated with late-onset AD, For example, the nucleotide pair (np) 4336 mutation in the $tRNA^{Gln}$. It has been observed that common 5-kb mtDNA deletion is elevated about 15-fold in AD patient brains. We analyzed 20 unrelated AD patients for mitochondrial mutation T4336C beside mutations in the complete regions of ND1, $tRNA^{Leu}$, ND2 and 16s rRNA by sequencing method. We also investigated common deletion in the blood samples of AD patients. The sequences were aligned upon the revised Cambridge Reference Sequence (rCRS) and any incompatibilities were recorded single base substitutions (SBS), insertions and deletions (Indels). The mtDNA A4336G mutation and mtDNA common deletion were not present in AD patient but our results revealed 6 new polymorphisms which had not been reported before in the mitochondrial databases. Our study suggests that the A4336G mutation were not associated with an increased risk of AD but other mtDNA mutations may contribute the risk factor to idiopathic AD.

P0630. Generation and characterization of recombinant hGSK-3β and hTau over-expressing EcR-293 cells

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The regulation of microtubule-associated protein Tau phosphorylation seems to be an important factor in the pathogenesis of Alzheimer's

disease. In vitro studies have shown that glycogen synthase kinase-3β (GSK-3β) plays an important role in the regulation of this process.
A cell-based assay has been developed to study GSK-3β-mediated Tau hyperphosphorylation in EcR-293 cell line. EcR-293 cells were co-transfected with human GSK-3β and human tau cDNAs using the inducible mammalian vectors. Cell colonies were tested for hGSK-3β and hTau mRNA as well as protein expression using quantitative RT-PCR and flow cytometry based immunocytochemistry. Protein expression was three- to fivefold over the basal level in the transfected and induced clones. Clone H11 exhibited the highest GSK-3β and tau levels; therefore it was chosen for investigation of tau phosphorylation using phosphorylation-site specific anti-tau antibodies.
In H11 cells pre-treated with the inducing agent muristerone A (MuA), a significant increase in tau phosphorylation at specific GSK-3β-dependent phosphorylation sites (Ser 202 and Ser 396) was detected. Flow cytometry analysis revealed a dose-dependent up regulation of tau hyperphosphorylation with increasing concentration of MuA. In addition, the GSK-3 selective inhibitor LiCl reduced tau phosphorylation in a concentration dependent manner.
Thus, the established cell line stably and inducibly co-expressing hGSKβ and hTau is a useful tool to investigate the effect of GSK-3β inhibitors on Tau phosphorylation.

P0631. Possible influence of oleic acid on fibrillogenesis of lysozyme

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In our study we research the influence of different substances on fibrillogenesis of model proteins such as human alpha-lactalbumin (hLA) and chicken egg white lysozyme (CEWL). There are lot of factors which can play role in fibrillogenesis in vivo but do not present in vitro. Fatty acids are one of these components. In silico analysis of CEWL structure lead us to model in which surface of fatty acid drop can take part in hiding of hydrophobic residues and increasing of local protein concentration to critical value. Oleic acid (C18:1) is known as agent that can interact with non-native form of hLA. So we decide to prove a role of oleic acid on initial stage of CEWL fibrillogenesis. CEWL(2 mg/ml) under denaturing conditions (pH 2.0, 100mM NaCl, 55 ºC) was covered with oleic acid drop. After 2 hours of incubation we observed a thin white film appearance on phase interface. We add Thioflavine T(ThT) to solution under oleic acid film. Fluorescent microscopy of 2 phase system including this film shows presence of ThT fluorescence on edges of oleic acid drops. Fluorescence of ThT is the evidence for amyloid fibrils presence. We do not observe any fluorescence on phase interface of control probe without any proteins or with bovine serum albumin. Role of fatty acids on initial stages of fibrillogenesis is discussed.

P0632. Ecogenetic nature of hypersensitivity reactions during hemodialysis

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Angiotensin I-converting enzyme inhibitors (ACEi) are associated with increased risk of hypersensitivity reactions (HSR) during hemodialysis using negatively charged membranes. We hypothesize that this side-effect of ACEi therapy is ecogenetic in nature. Our study measured aminopeptidase P (APP) activities in the plasma of 14 patients who suffered an HSR (HSR+) and control group (n=39) who did not develop any side effect (HSR-) during dialysis with an AN69 membrane while simultaneously treated with an ACEi. We hereby report a significantly decreased (p=0.013) plasma APP activity and an altered degradation (lower β value, p<0.001) of endogenous des-Arginine[9]-Bradykinin among HSR+ subjects. The same analytical approach was also applied to 171 relatives of the HSR+ patients. Variance component analysis using these 14 families suggests that genetic differences may explain 61% of the phenotypic variability of plasma APP activity (p<0.001). Furthermore, we show that a polymorphism (C-2399A) at the *XPNPEP2* locus is a significant predictor of APP activity among the HSR- controls (p=0.029). In addition, there was a significant difference in mean APP activity by genotype (p<0.001), using a recessive genetic model for the A allele. Finally, our results define the non-specific inhibition of recombinant APP by some ACEi drugs. In conclusion, our study highlights the complexity of HSR, its multifactorial nature and expression of genetic variation in response to the environment and drugs, suggesting an ecogenetic character for these rare, but life-threatening adverse events.

P0633. Association between APOA5 -T1131C mutation and triglyceride level in Hungarian patients with metabolic syndrome and diabetes mellitus

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Objectives: The T>C SNP in the promoter region of the apolipoprotein A5 (apoA5) gene at position -1131 is strongly associated with elevated plasma triglyceride level (TG); thereby the C variant means a risk factor for cardiovascular disease (CVD). The prevalence of ischemic cardiovascular disease is known to be higher amongst metabolic syndrome patients and patients with diabetes. Patients and methods: We studied the distribution of this substitution using a PCR-RFLP assay in 120 Hungarian patients with metabolic syndrome, 68 with type 2 diabetes mellitus and 280 non-diabetic controls. Results: The level of TG was increased in the metabolic syndrome patients (2.99±0.30) and in diabetic patients (2.21±0.15) compared with the controls (1.29±0.64 mmol/l; means ± SE, p< 0.05). The frequency of the C allele was also increased in patients with metabolic syndrome (13.4%) and diabetic subjects (14%) compared with the controls (5%). Conclusions: Our results suggests that increased prevalence of the apoA5 T1131C variant in metabolic syndrome and diabetic patients can be a cause of the increased CVD incidence rate in these diseases via the increased TG levels.

P0634. Apolipoprotein E gene polymorphism and plasma lipid levels in obese and dislipidemic Turkish children

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The aim of this study was to search the relation between apolipoprotein E gene polymorphism and plasma lipid levels in obese and nonobese Turkish children and apoE gene polymorphism in dislipidemias coexisting with obesity.
The study population was composed of genetically nonrelated children who were having primary obesity in 57 and nonobese 18. In obese children apo A1 levels were higher. In obese and nonobese group, the distrubition of E2/E3 was 10,5%, 27,8% ; E3/E3 80,7%, 61% ; E3/E4 7%, 5,6% respectively. E2/E4 was 1,8% in obese group and E4/E4 5,6% in nonobese group. Allele frequency in obese and nonobese group was 6,1%, 14% for epsilon 2 (ε2) allele, 89,5% and 7,8% for epsilon 3 (ε3) allele, 4,4% and 8% for epsilon 4 (ε4) allele. In obese children, ApoB level was higher in group E3.
The distribution of Apo E allele and phenotype in obese and healthy children was similar. Apo E phenotype distribution of study population showed similarity to Mediterranean societies.
In obese group total cholesterol, Apo B, Apo A1 level found to be higher. We determined that 44% of the obese children had hyperlipidemia, and in comparison with the nonobese children, we found higher levels of ApoB which had atherosclerotic property and Apo A1 which had anti-atherosclerotic effects in obese group. In the nonobese healthy children, mean total cholesterol and LDL levels were higher in group E4 than group E3 and E2.

P0635. High resolution analysis by X chromosomal array comparative genomic hybridisation (array-CGH) in patients with XLMR

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The molecular basis of the X-linked metal retardation (XLMR) is poorly understood due to the hight genetic heterogenity of this disorder; nowadays OMIM lists 359 entries about XLMR although only 44 X-linked genes are known to cause XLMR (syndromic and non-syndromic). In order to screen for submicroscopic aberrations in patients afected by XLMR, we have constructed a full coverage X chronosome BAC array for comparative genomic hibridization (array CGH) that consists of 1,600 genomic clones derived from the X chromosome. The sensitivity and specificity of the array were tested in a series of normal versus normal experiments and a series of patients with known chromosome X copy number aberrations (including an Xp11.4 deletion, Xq22-q26 duplication and Xp11-p21 duplication). The presence of DNA copy number aberrations in XLMR was investigated in a cohort of 30 XLMR patients. Preliminary analysis detects. a) Two deletions: one affecting OPHN1 gene (Xq12) and the other affecting FLNA, EMD and TAZ genes (Xq28). b) 9 duplications:7 cases harbouring segmental duplications in Xq26.2 and Xq28, one duplication affecting NXF3 and BEX1 genes (Xq22.1) and other affecting EDA2R gene (Xq12). Confirmation by others methods and clinical and molecular characterization are being performed. Array CGH assays has become a powerful tool for the genome wide screening for copy number aberration and will facilitate the diagnosis and identification of novel genes involved in X-linked mental retardation. (FIS 04/1126, V2003-REDC-07, REDG-098)

P0636. Investigation of mitochondrial deletions and Haplogroups in Persian Ataxia-Telangiectasia patients

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Ataxia-Telangiectasia (AT) is a rare human neurodegenerative autosomal recessive multisystem disease that is characterized by a wide range of features including, progressive cerebellar ataxia with onset during infancy, occulocutaneous telangiectasia, susceptibility to neoplasia, occulomotor disturbances, chromosomal instability and growth and developmental abnormalities. Mitochondrial DNA (mtDNA) has the only non-coding regions at the displacement loop (D-loop) region that contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. We investigated mt-DNA deletions and haplogroups in AT patients. In this study, 24 Iranian patients affected with AT and 100 normal controls were examined. mt-DNA was extracted from whole blood and examined by 6 primers for existence of any mitochondrial deletions. We also amplified and sequence the mtDNA HVS-I by standard sequencing techniques. mtDNA deletions were present in 13 (76%) of our patients (9.0 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. Our results may explain involvement of mitochondrial damage in the pathogenesis of the AT

P0637. Molecular Diagnostics for Autosomal Recessive Polycystic Kidney Disease.

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Autosomal Recessive Polycystic Kidney Disease (ARPKD) is a severe form of polycystic kidney disease characterized by enlarged kidneys and congenital hepatic fibrosis. It is caused by mutations in the Polycystic Kidney and Hepatic Disease 1 (*PKHD1*)-gene, which consists of 86 exons that are variably assembled in a number of alternatively spliced transcripts. Mutation analysis was performed by direct sequencing of the 67 exons encompassing the largest open reading frame. So far, in 39 families the following mutations were identified: 11 nonsense mutations, 15 deletions/insertions, 5 splice site mutations, and 39 missense mutations. To classify missense variants we combined evolutionary conservation, using the human, chimpanzee, dog, mouse, chicken and frog Pkhd1 sequences, with the Grantham score for chemical differences. Thirty-three missense mutations were considered pathogenic and 7 were classified as rare, probably pathogenic variants. In 31 index patients two mutations were found, in 6 patients one mutation was found, leading to a mutation detection rate of 87%. More families are currently under investigation. The analysis of amino acid conservation as well as applying the Grantham score for chemical differences allowed us to determine the pathogeneity for nearly all new missense mutations and thus proved to be useful tools to classify missense variants.
In addition to sequence analysis, MLPA was used to identify multiple exon deletions. We selected 9 probes equally spread over the gene but did not find deletions, indicating that large deletions in the *PKHD1* gene are not a frequent cause of ARPKD.

P0638. Mutational analysis of the *BBS2* gene involved in Bardet-Biedl syndrome in Spanish patients

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Bardet-Biedl syndrome (BBS; OMIM 209900) is a heterogeneous pleiotropic human disorder with the main clinical features of retinopathy, obesity, polydactyly, hypogenitalism, mental retardation and renal abnormalities. These patients have an increased incidence of diabetes mellitus, hypertension and congenital heart malformations. Although BBS is rare in the general population (about 1/160,000 in West Europe), there is a considerable interest in its study because the components of its phenotype are very common. Until now, nine involved genes have been identified (*BBS1-9*) and several pieces of evidence have suggested their role in ciliary function. BBS is a complex inheritance disease and it has been proposed as a triallelic trait in some families, where three mutated alleles in two genes are necessary to exhibit the phenotype. The *BBS2* gene has 17 exons and is situated on 16q21. *BBS2* displays a wide pattern of tissue expression, however there is no information available regarding the specific function of this gene. We analyzed the involvement of mutations in *BBS2* for 44 patients by PCR-SSCP. An abnormal pattern of SSCP was detected in exons 2 and 3. The results show an implication of *BBS2* in less than 5% of our pool of patients. It has been described that 40% of the BBS2 families presents mutations in other related genes. The patients with mutations in the *BBS2* gene lack mutations in *BBS1* and *BBS6* genes, previously analyzed by some of the authors, suggesting that the triallelic inheritance is not present in the families studied.

P0639. Functional analysis of a novel KCNQ2 gene variant found in a large pedigree with benign familial neonatal convulsions (BFNC).

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Benign Familial Neonatal Convulsions (BFNC) is a rare epilepsy disorder with autosomal dominant inheritance. Mutations in the voltage gated potassium channel genes KCNQ2 and KCNQ3 are highly associated with BFNC. KCNQ2 and KCNQ3 subunits form functional tetrameric voltage gated channels. Assembly of the conserved A-domain located in the carboxy-terminus of the proteins, results in a classical M-current. In many neurons, the M-current is important in regulating excitability and in reducing repetitive action potential discharges. Previously, we have reported a splice-site mutation in the KCNQ2 gene in a BFNC family with 13 affected members. This mutation (c.IVS14-6(C>A)) creates a new, preferentially used, splice site, that results in a premature stop codon and a predicted protein truncation (p.R588X). Here we report the biophysical properties of this

mutant. Whole-cell voltage clamp analysis reveals the presence of a large M-type potassium current in transfected HEK293 cells which overexpress wild-type KCNQ2/KCNQ3 heteromeres. In contrast, cells transfected with KCNQ2/KCNQ3 and mutant KCNQ2 cDNA in a 1:2:1 ratio, show a large reduction in M-current (wild-type 781±170 pA/pF (n=7); mutant 224±53 pA/pF (n=7)). Furthermore, the voltage dependence of channel activation in cells expressing the mutant is shifted in the depolarizing direction by 6mV. These changes may result in hyper-excitability of neurons in which the M-current is important in modulating the resting membrane potential.

P0640. Gender Difference in the DAT1 -67 T-Allele Homozygosity and Predisposition to Bipolar Disorder

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BACKGROUND: Linkage and association studies implicate the dopamine transporter gene (DAT1) in the etiopathophysiology of bipolar disorder. We have recently reported the association between the DAT1 core promoter -67A/T polymorphism and this disorder in a sample of Iranian patients. For the first time, these data support sex difference in the homozygosity for the -67 T-allele between male and female affected cases. METHODS: The present study was undertaken with a larger sample size of cases (N = 240) and controls (N = 213) to determine whether there is consistent difference between male and female patients and homozygozity for this allele. RESULTS: The results are consistent with our preliminary observation that homozygosity for the T-allele is a predisposing factor in male patients, but not in females (χ^2= 8.825, df = 1, $p \leq 0.003$, 95% CI, OR = 3.624). CONCLUSION: This finding may reflect one of the proposed neuroprotective effects of estrogen upon the negrostriatal dopaminergic system through antagonizing the dopamine transporter protein in females.

P0641. BMP4 expression and it's role in the rib cage development

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Background: Recent data shows that the Bmp4 plays significant roles in a large number of developmental processes, including bone formation and development.

Objective: The purpose of this study was to identify the Bmp4 expression and its role in the development of ribs and sternum and analysis of the skeletal phenotypes caused by the genetic inactivation of Bmp4. along with the study of it's expression patterns.

Design and Methods: Bmp4 lacZ mice, where expression of the inserted lacZ is controlled by the entire endogenous Bmp4 gene, were used for mapping all Bmp4 expression domains in the bones that form the thorax.

To examine the onset of endogenous Bmp4 expression in ribs , heterozygous Bmp4 lacZ newborn mice (PN1) were utilized for analysis of ß-gal activity.

B-Galactosidase staining was assessed in newborn mice using newborn mice who were fixed with ice-cold 4% paraformaldehyde for 30 min to 1 h, and then washed three times with PBS for 5 min each. The specimens were then stained overnight in freshly made X-Gal solution (1 mg/ml) at 32^0 C.

Results: Asynchronous ossification of inferior sternal segment 5 was recorded .This ossification segment differed from all the other four centers and had a modified shape . We have noticed that in some cases this center was redused in size and in others fused with segment 4 giving it an unusual shape.

Conclusions: These results indicate that Bmp4 gene dosage is essential for the normal development of the sternal bone and ribs .

P0642. Identification of the mutation in the Receptor Tyrosine Kinase Gene *ROR2,* which causes Brachydactyly type B1.

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Brachydactyly type B1 (BDB1) is an autosomal dominant skeletal disorder characterized by abnormal development of the distal phalanges and nail dysplasia. This disorder is caused by mutations of the orphan receptor tyrosine kinase ROR2 that is encoded by the *ROR2* gene (9q22). ROR2 consists of extracellular immunoglobilin-like, cysteine-rich, kringle, transmembrane domains and cytoplasmatic tyrosine kinase domain, distally located serine-threonine-rich and proline-rich domens. Only mutations flanking tyrosine kinase domain result in BDB1 phenotype, in all other mutations localization the autosomal recessive Robinow syndrome develops. We have investigated one BDB1 family in which two affected members had typical features of diseases. DNA sequence analysis of *ROR2* gene exons concerned with BDB1 revealed heterozygous 2-bp deletion in exon 9. This mutation led to frame shift due to 1397-1398 delAA and located proximal to the tyrosine kinase domain. The 1398insA mutation in the same position has been reported previously.

P0643. Novel application of SNPlex Genotyping assay for breast cancer mutation screening

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There is widespread pressure for genetics laboratories to undertake faster mutation screening of the full BRCA1 and BRCA2 genes in patients with a family history of inherited breast/ovarian cancer. We will achieve this using a pre-screen approach combining the SpectruMedix Temperature Gradient Capillary Electrophoresis (TGCE) Reveal system to detect point mutations by heteroduplex analysis followed by sequence analysis to characterise them and MLPA analysis to detect whole exon deletions and duplications. A consequence of this screen will be the identification of a large number of SNPs (147 SNPs are recorded in the databases in 83 BRCA amplicons). Our solution to reduce the amount of sequencing is to utilize the Applied Biosystems SNPlex Genotyping System 48-plex assay to genotype all our patients for 45 of the most common SNPs and three most common Ashkenazi Jewish mutations. After mutation analysis amplicons would be prioritised for sequencing if they have a heteroduplex but are not heterozygous for a SNP. SNPs were selected if they had a heterozygosity >3% and only one SNP was chosen from SNPs known to be in linkage disequilibrium. The SNPlex assay uses oligonucleotide ligation assay combined with multiplex PCR to discriminate between alleles and target amplification, while GeneMapper software provides automated data processing. This highly automated technology will allow us to genotype 384 samples in 72 hours.

P0644. A novel Notch3 gene mutation in a patient with CADASIL from southern Italy

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Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is a late-onset syndrome characterized by subcortical ischemic strokes, attacks of migraine with aura, and vascular dementia. Subcortical dementia, in all cases associated with pseudobulbar palsy, is the second commonest clinical manifestation of CADASIL. Epilepsy has been reported in 2-10% of patients, most often following strokes. All individuals, both symptomatic and asymptomatic, have prominent signal abnormalities on brain Magnetic Resonance Imaging (MRI).

CADASIL is caused by single missense mutations, small in-frame deletions, or splice site mutations in the Notch3 gene encoding a transmembrane receptor. All previously reported mutations resulted in an odd number of cysteine residues within one of the 34 epidermal growth factor (EGF)-like repeats in the extracellular amino-terminal region of the Notch3 receptor.

Here we report the apparently sporadic case of a 65 years-old woman with moderate cognitive deficit and diffuse leukoencephalopathy at the MRI scans. The evaluation of Notch 3 gene revealed a novel mutation in exon 11, located within the extracellular domain of the gene and predicted to result in a gain of a cysteine residue in position 574. As this mutation segregates with the neurologic and clinical phenotype, we suggest that it is the disease causative mutation in our patient.

This study provides the first evidence of a mutation in Notch3 gene exon 11 in a subject from southern Italy. Furthermore, the finding of a new mutation contribute to enlarge the spectrum of Notch3 gene mutations.

P0645. Molecular findings in *CAPN3*, *DYSF* and *GNE* related to various muscle diseases

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The identification of mutations in large-sized genes is challenging on a routine basis in clinical diagnostic settings. Methods for mutation screening are particularly adapted to this task.

Here, we report our experience in mutation screening using DHPLC analysis and subsequent sequencing of detected variants, in the large-sized genes encoding calpain-3 (*CAPN3*, 15q15.1-q21.1, 24 coding exons), dysferlin (*DYSF*, 2p13.1-p13.3, 55 coding exons) and UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (*GNE*, 9p12-13, 11 coding exons), in three cohorts of respectively 48, 93, and 31 myopathic patients.

Mutations in *CAPN3* and *DYSF* cause the most frequent forms of autosomal recessive Limb Girdle Muscular Dystrophies (LGMD2), respectively LGMD2A and LGMD2B. The most common form of autosomal recessive (AR) hereditary inclusion-body myopathy (HIBM) is caused by mutations in *GNE*.

Patients were included after initial clinical and pathological assessment, allowing to select the subsequent molecular analyses. A resume of the results of the molecular analyses are presented in the table below. At least one pathogenic allele was detected in >85% of patients included for *CAPN3* and *DYSF* analysis. The lower mutation detection rate observed for *GNE* may be correlated to genetic heterogeneity and/or difficulties in defining phenotypic criteria for the inclusion of patients. Mutations identified in the *CAPN3* and *DYSF* genes were compiled in the locus-specific databases UMD-CAPN3 and UMD-DYSF, adapted from the generic software UMD (Universal Mutations Database).

P0646. Functional analysis of new Blau syndrome-associated CARD15 mutations.

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Blau ssyndrome (BS) is a very rare autosomal dominant syndrome characterized by early onset granulomatous arthritis, uveitis and skin rash, caused by high penetrance mutations in the CARD15 gene encoding a cytoplasmic receptor of bacterial peptidoglycan natural immunity . In contrast with the common Crohn's disease-associated SNPs, which are located in the final ligand binding domain and impair ligand recognition, the identified Blau s. mutations are all in the central nucleotide binding domain and may activate ligand independent signals, in accordance with the lack of intestinal involvement.

In an Italian BS family we recently described the CARD15 E383K mutation (van Duist et al. 2005). The position in the NBD close to a magnesium binding site, its segregation with the disease, its absence in 100 healthy controls and its conservation in other NBD containing genes and in homologous genes of different species, suggested this mutation to be pathogenic.

Here we report the functional analysis of this variant by a gene reporter assay, that proved this mutation to be gain-of-function. The E383K variant showed a 3 fold increased NF-kB activity compared to the wild type protein, slightly lower than the previously identified mutations (R334Q/W, L469F = 4 fold). Similar investigation is underway for another never earlier described NBD variant W490L we identified in a BS affected child born from healthy parents (Patient from A.Martini and M.Gattorno, Gaslini Institute, Genova).

P0647. Biochemical and genetic analysis of Spanish patients with congenital disorder of glycosylation

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Congenital disorder of glycosylation (CDG) is a heterogeneous genetic group of metabolic inherited disorder, clinically characterized by a central nervous system dysfunction and multiorgan failure. Most of these disorders are associated with several enzymatic defects involved in N-glycosylation of proteins. We present the biochemical and genetic study of a cohort of 17 affected patients identified using % CDT (carbohydrate-deficiency trasferrin) level and typical isoelectric focusing pattern. We have identified six patients belonging to the most common type CDG-Ia and one patient CDG-Ib. Up to now the remaining patients are still unclassified. Fibroblasts phosphomannomutase (PMM) activity from the six CDGIa patients ranged between 0.8-2.3 mU/mg (control range 6.5±1.7). Eleven allelic variants of the 12 mutant alleles analyzed have been identified by sequencing of the entire coding region of the PMM2 cDNA and the corresponding gDNA regions. We have identified eight different mutations, two of them are novel, one being a missense change (D209G) and the variant allele IVS7-9T>G located in the polypirimidine tract (U) of last intron of PMM2 gene. The T to G change in the U tract of intron 7 likely disturbs the binding site of the splicing factor U2AF. We have also identified one CDG-Ib patient bearing the already described change R219Q in MPI gene. The residual mannose phosphate isomerase activity measured in patient's fibroblasts was 2.1 mU/mg (control range 12.8±2.7). Based on the clinical, biochemical and genetic results, we will discuss the phenotype-genotype correlation.

P0648. The investigation of CFTR gene mutations in cohort of infertile men from Ukraine

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It was supposed that apart from cystic fibrosis, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are also involved in male infertility. To study the possible association between CFTR gene mutations and spermatogenesis we have analyzed the delF508(10exon), R117H(4exon), 621+1G-T(4exon), N1303K(21exon), CFTR2,3del21kb, P1290S(20exon) mutations and poly -T sequence (8 intron) in the group of 365 patiens with azoospermia and oligospermia(involved in ICSI programme), none had pulmonary or gastrointestinal manifestations of cystic fibrosis and in 474 healthy valantiers. The CFTR gene mutations were analyzed by PCR-based methods, poly-T alleles were determind by fluorescent-sequencer (ALF express) method. Nobody from analyzed patients determined as a carrier of AZFa, AZFb and AZFc regions (long arm chromosome Y). 5T allel (8intron) of CFTR gene associated with CBAVD was detected in 5.3% in our group of patients.The same frequency of 5T allel was detected in common populations of different countries. The frequency of CFTR gene mutations carriers detected in our group of patients (8.22%) was statisticaly significant higher (P=0.017) than in control group (2.53%). More over, R117H mutation identified in the group of infertile men was revealed neither in cystic fibrosis patiens nor in control group from Ukraine.

The obtained data produce the evidence of the possible involvement of CFTR protein in spermatogenesis. To specify the association between CFTR gene mutations and pathogenesis of spermatogenesis the other CFTR gene mutation and polymorphisms analysis in unfertile men require continued practical and theoretical research.

P0649. Y chromosome microdeletions and CFTR gene mutations in Serbian infertile men

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The most frequent genetic causes of male infertility are considered to be Y chromosome microdeletions and mutations in the Cystic Fybrosis Transmembrane Conductance Regulator (CFTR) gene. This study has encompassed 33 infertile men (10 with obstructive azoospermia,

11 with impaired spermatogenesis or sperm maturation and 12 with unknown cause of infertility) who were screened for the presence of Y chromosome microdeletions and CFTR gene mutations. The presence of microdeletions in the AZF region of Y chromosome was analyzed by multiplex PCR analysis. The screening of the CFTR gene was performed by denaturing gradient gel electrophoresis (DGGE) method. The presence of the 5T allele of CFTR Tn polymorphism was analyzed by PCR-mediated Site-directed Mutagenesis (PSM) method. Deletions on Y chromosome were detected in 4 patients. Three CFTR mutations (F508del, 711+3A/G and D1152H) were detected on 8 chromosomes, while the presence of 5T variant was detected on 6 out of 66 analyzed chromosomes. The presence of Y microdeletions and/or CFTR mutations was detected in 6 of 11 patients obstructive azoospermia, in 3 of 12 patients with impaired spermatogenesis or sperm maturation and in 3 of 13 patients with unknown cause of infertlity. This study has confirmed that both Y chromosome microdeletions and CFTR gene mutations play important roles in etiology of male infertility, as well as that they are found more often in men with more severe spermatogenic defect.

P0650. Multiplex polymerase chain reaction (PCR) of short tandem repeats analysis of chimerism after allogeneic bone marrow transplantation

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Monitoring the engraftment of donor cells after allogeneic bone marrow transplantation (allo-BMT) is important for the early diagnosis of graft failure or relapse of disease. Short tandem repeats (STRs) as highly polymorphic DNA sequences in the human genome have been used for monitor BM engraftment after allo-BMT. Engraftment analysis requires one or more informative STR loci that distinguish recipient from donor.

For monitoring of the engraftment, a quantitative, non-isotopic method using PCR of STR marker has been started up in our laboratory of Biomedical Research Centre in July of 2005.

DNA samples from pretransplant recipient's and donor's peripheral blood were amplified with the *AmpFlSTR Identifiler PCR Kit,* which contains 16 STR markers and PCR products were analyzed in ABI PRISM 310 Genetic Analyzer. Informative STR alleles which can distinguish donor from recipient have been selected as markers. Posttransplant DNA genotypes of recipients have been analyzed by using DNA from peripheral-blood and BM samples.

159 PCR-STR analyses had been carried out during July-December of 2005. Were monitored 36 patients after allo-BMT (88 PCR-STR analyses). Complete chimerism has been detected in 20 patients, fluctuations of chimerism status in 8, mixed chimerism - in 7. Donor cells were absent only in one patient after 40 days of allo-BMT.

In summary, this method provides an accurate, versatile, quantitative, and early assessment of mixed chimerism in posttransplant patients. Such information may be useful to guide implementation of additional treatment to circumvent graft failure or relapse in future.

P0651. Novel nonsense REP-1 R270X mutation in a familial case of choroideremia

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Choroideremia (CHM; MIM #303100) is an X-linked recessive progressive chorioretinal degeneration. Night blindness and progressive loss of peripheral vision become evident by the second and third decade of life in most patients with progression to tunnel vision or complete blindness by middle age. The disease results from mutations in the CHM gene, located in Xq21. The product of this gene, Rab escort protein (REP)-1, is involved in the posttranslational lipid modification and subsequent membrane targeting of Rab proteins, small GTPases that play a key role in intracellular trafficking. All mutations in CHM result in the truncation or absence of the normal protein product. We describe the clinical and molecular analysis of a Mexican family with CHM including 3 affected males, 2 obligate carrier females, 6 asymptomatic females and 4 asymptomatic males. The ophthalmologic examination included best-corrected visual acuity, color vision tests, slit-lamp examination, fundus examination, Goldmann visual fields, electroretinography, and fluorescein angiography. To detect mutations of the CHM gene, all 15 exons were amplified by PCR and the products directly sequenced. Fundus examination in affected males revealed mottling of the RPE without macular involvement typical of CHM. There was evidence of differences in fundus findings between some carrier females. CHM gene analysis revealed a novel C to T mutation at nucleotide 838 in exon 6, which predicts a R270X nonsense mutation. This is the first description of molecular analysis in Mexican patients with CHM; our results expand the mutational espectrum of the CHM gene in choroideremia.

P0652. Genetic testing in Czech patients with ‚idiopathic' and hereditary chronic pancreatitis

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The aim of our study was to evaluated the profile of genetic mutations in patients with hereditary and idiopathic chronic pancreatitis and to compare it with the healthy controls in the Czech population.

Methods: We have screened for common *PRSS1* (R122H, N29I), *SPINK1* (N34S) mutations in a total of 124 chronic pancreatitis (CP) cases ("idiopathic" CP-ICP /incl. criteria: *N Engl J Med* 1998, 339: 653; 46 children,49 adults/; "hereditary" CP- HP /EUROPAC incl. criteria: *Med Clin North Am* 2000, 84: 575; 11 children, 18 adults/ and compared their frequency to 227 random controls /112F, 115M; age range 18-45 years./).

Results: The frequency of R122H and N29I *PRSS1* mutations was significantly increased in both children/adults (8/58) with HP ($p < 0.001$), but not in all ICP cases (2/190) compared to controls (0/454). However the *SPINK1* N34S mutation was found at both HP (4/58) and adults ICP, the frequency was increased only in children (8/92) with ICP ($p < 0.001$) compared to controls (8/454 chromosomes).

Conclusions: Our data indicate that mutations in *PRSS1/SPINK1* genes are associated with ICP /HP.In cases with positive screening results genetic counseling and long-term monitoring for the development of the adenocarcinoma of pancreas (in HP) should be provided. *Supported by MZCR: 000000064203; MSMT: #111300003.*

P0653. Twirler, human chromosomal rearrangement, adhesion molecules and cleft palate.

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In 1958 Lyon first described the spontaneous semidominant mutation "twirler" in mice presenting craniofacial and inner ear malformations. Ting et al. (1993) found equivalent phenotypic traits in mice with the transgene-induced mutation 9257, and they managed to map the Tg-9257 region to human 18q11.

We present a proband with unilateral cleft lip and palate, congenital cataract, and locomotor clumsiness, 46,XX,t(8;18)(q2?;q11.2). The breakpoint on chromosome 18q11.2 has been mapped down to 150kb. Within the 2,5Mb surrounding this breakpoint, two candidate genes have been described (in both human and mouse): LAMA3 gene, coding for α3 chain of the adhesion molecule Laminin-5, which strongly promotes adhesion, migration, and scattering of cells through binding to integrins α3β1, α6β1 and α6β4; and ROCK1 gene, coding for a Rho-associated coiled-coil forming kinase, involved in integrin and integrin-associated structure organization.

It seems possible that the chromosomal localization of these two genes might be important for their expression, and so a chromosomal rearrangement involving the region between them could affect their transcription. If so, the translocation identified in proband B2157 that separates LAMA3 and ROCK1 genes, could be the cause of a reduced adhesion capacity of the epithelial cells, which are directly involved in the adhesion and fusion of the palatal shelves in the formation of the secondary palate. The consequence of this lack of adhesion and fusion would be the appearance of a cleft palate.

P0654. Early onset X-linked Charcot-Marie-Tooth disease. Two novel mutations in the GJB1 gene one associated with abortion

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X-linked Charcot-Marie Tooth (CMT) is caused by mutations in the connexin32 gene (GJB1). The gene encodes a polypeptide which is arranged in hexametric array and form gap junctions. We describe two novel mutations in the connexin32 gene in two Norwegian families. Family 1 had a 225delG which cause a frameshift and premature stop codon at position 247. This probably results in a polypeptide which is not arranged in a hexametric array due to the change in protein structure. This family had a female preponderance of X-linked CMT and several abortions were observed. Family 2 had a 536 G→A transition which cause a change of the highly conserved cysteine residue to tyrosine at amino acid position 179. One man had pathological VEP and three affected had markedly balance problems of probable central nervous system origin. The mean age at onset was in the first decade in both genders. Clinically the affected had symmetrical findings, while the neurophysiological examination revealed minor asymmetrical findings in nerve conduction velocity in 6 of 10 affected. We conclude that the two novel mutations in the connexin32 gene are more severe than the majority of previously described mutations.

P0655. Charcot-Marie-Tooth syndrome. Two novel mutations in the myelin protein zero (MPZ) gene

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Charcot-Marie-Tooth (CMT) disease is characterized by distal muscle wasting and weakness, sensory loss with reduced tendon reflexes and foot deformity. It is the most common inherited disorder of the peripheral nervous system with an estimated prevalence of 1 in 2,500. CMT is a heterogeneous disorder with respect to clinical features, neurophysiology, pathophysiology and genetics. The number of identified CMT genes is still expanding. So far the majority of the CMT genes encodes either neuronal or Schwann cell proteins. The myelin protein zero (MPZ) gene is expressed in the compact layer of the Schwann cells. We present two new mutations in the MPZ gene as well as the clinical features in the affected Norwegian families.

P0656. Detection of CMTX in an Iranian family

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The Charcot-Marie-Tooth (CMT) syndrome is a heterogeneous group of neurological disorders that produce hereditary peripheral neuropathy associated with symmetrical distal muscle weakness and atrophy, sensory loss, and frequently depressed tendon reflexes, high arched feet, and abnormal electrophysiological testing. Approximately one in every 2500 people is affected and the onset of the disease usually occurs in the second or third decade of life. Some classifications are based on the type of the neuropathy, which may be axonal or demyelinating, and on the mode of inheritance.
Electrophysiological studies are necessary to determine the type of neuropathy. Different modes of inheritance are observed: autosomal dominant, X-linked, dominant, autosomal recessive.
The X-linked form of CMT (CMTX) is associated with mutations in the connexin 32 (Cx32) gene, which maps to chromosome Xq13. Xq13s are often reduced in CMTX males (< 40 m/s), but ranged from slightly reduced to normal values in females. Neuropathy in CMTX is primary axonal or demyelination with secondary axonal degeneration. CMTX involves defective Schwan cell function.
So far, 33 kinds of mutation in 30 sites in the Cx32 gene have been found in CMTX disease patients.
CMTX disease-associated Cx32 gene mutations are not only unable to restore GJIC but also down-regulate wild-type Cx32 protein functions. In our study we detected a family with 3 males and 1 female affected CMT. Reviewing the pedigree and electrophysiological studies showed that this disorder is suspected to be X-linked CMT. Therefore we checked Cx32 by PCR method and sequencing for confirming our detection.

P0657. PCR-based diagnosis of CMT1A duplication and HNPP deletion in Belarus

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Charcot-Marie-Tooth disease type 1 (CMT1) is the most common hereditary peripheral neuropathy, occurring with a prevalence of ~1 in 2500. About 70% of individuals with CMT1 have a duplication of PMP22 gene as the origin of disorder (CMT1A form). One of the most effective strategies for molecular diagnosis of CMT1A is based on quantitative PCR analysis of short tandem repeats (STRs). To establish the diagnostic potential of this method we used 3 STR markers, from within the CMT1A duplicated region - D17S2218, D17S2223 and D17S2229. A multiplex PCR protocol was developed to amplify simultaneously all 3 dinucleotide repeats. Products were analysed by automated capillary electrophoresis on the ABI Prism 310. First we amplified these sequences in a cohort of unaffected individuals from Belarus population and assessed the heterozygosity and number of alleles for each of STR. Heterozygosity values of the selected STRs were 78%, 65 % and 76% respectively. Then markers informativeness was tested in a group of 58 patients with presumable clinical diagnoses of CMT neuropathy and 68 their family members. We identified the PMP22 gene duplications in 9 families with a total number of 20 affected individuals. In all positive cases we were able to detect three different alleles of at least one STR and clear semiquantitative dosage effect in other. Additionally one family with hereditary neuropathy with liability to pressure palsy (HNPP) was identified. This is the first experience of CMT1A diagnosis in Belarus, which show fidelity and applicability of quantitative PCR-based analysis.

P0658. Polymorphism of the five new STR markers within the 17p11.2 genomic region in Russian Siberian population and its application for molecular diagnosis of CMT1A

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Hereditary motor and sensor neuropathies (Charcot-Marie-Tooth disease) is a heterogeneous group of disorders of which CMT type 1A is the most common, and more than 70% cases of CMT1 are associated with 1,5-Mb duplication in 17p11.2. With an aim to estimate the polymorphism of new potentially more informative for molecular diagnosis STR markers within the 17p11.2 genomic region in Russian Siberian population we studied 5 STRs with tri- (D17S2228), tetra- (D17S2224, D17S2226) and pentanucleotide repeates (D17S2227, D17S2230) [Badano J. et al, 2001]. Allele typing was performed using PCR and subsequent high-resolution PAAG electrophoresis. We have analysed 107-119 DNA samples from unrelated subjects and observed 6 alleles (187-207 bp) of D17S2224 locus, 8 alleles (116-144 bp) of D17S2226 locus, 10 alleles (239-284 bp) of D17S2227 locus, 5 alleles (164-176 bp) of D17S2228 locus, 17 alleles (217-297 bp) of D17S2230 locus, and observed heterozygosities were 0.730, 0.826, 0.763, 0.626, and 0.826, respectively. Genotype frequency distributions were consistent with HW equilibrium in all groups. According to our results in Russian Siberian population the polymorphic potential of D17S2230, D17S2226, D17S2227, D17S2224 markers are the most useful for molecular diagnosis of CMT1A in Siberian region. The 5 STR marker panel was greatly informative in Russians and was applied for analysis of 17p11.2 duplication in CMT patients. Their use allows to increase reliability of DNA-diagnosis in families. We present our first experience (3 cases) in prenatal diagnosis of CMTA1 performed in Siberian region (100% information). These markers provide new tools for efficient diagnosis of CMTA1.

P0659. Polymorphic short tandem repeats for diagnosis of the Charcot-Marie-Tooth 1A duplication.

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Charcot-Marie-Tooth disease (CMT) and hereditary neuropathy with liability to pressure palsies (HNPP) are the most frequent inherited disorders of the peripheral nervous system. They are clinically and genetically heterogeneous. Molecular genetic studies have made major breakthroughs in unraveling the underlying gene defects, and DNA diagnosis can now be offered to a large number of families with distinct forms of hereditary peripheral neuropathies.

58 families with increased risk of autosomal dominant CMT1 passed clinico-neurological, electrophysiological examination, and molecular study, which were included in long-term registry of Moldova. Some genetics features of the disease have been revealed (intrafamilial polymorphism of different degree, effect of the ancestor in the dominant forms). Electrophysiological peculiarities of the separate clinical-genealogical variations of the pathology were found. Most of this families were Moldavian by origin. We test 3 STRs located within the duplication (D17S921, D17S122, D17S834). STRs were selected and used to test a set of 99 unrelated CMT1A patients and were compared with nonduplicated controls.

The CMT1A duplication was determine in 68.75% cases (gene dosage for heterozygous samples- different fluorescent intensity and /or three alleles. The most informative STR in our group was D17S834 locus-37.6%, and the less informative was D17S921-27%. Using 3 markers instead of two, which we used in our practices a few years ago allow to increase the determination of duplication.

Conclusion: Combined use of the three STRs allows robust diagnosis with almost complete informativeness. In our routine diagnosis for CMT1A, they have replaced the use of other polymorphic markers.

P0660. Molecular analysis of the *COH1* gene in Cohen syndrome.

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In 1973, Cohen et al. described a new syndrome whose main features were truncal obesity, hypotonia, mental retardation of variable degree, characteristic craniofacial dysmorphisms (down slanting palpebral fissures, short philtrum, open mouth, prominent upper central incisors, prominent nose) and abnormalities of the hands and feet. Beside the facial gestalt, major diagnostic criteria of Cohen syndrome include retinal dystrophy and neutropenia. This syndrome is transmitted as an autosomal recessive trait, with considerable variability of expression. Recently, mutations in *COH1* gene (locus 8q22-q23) have been reported in patients with Cohen syndrome.

We have collected a cohort of 21 patients, originating from different countries. A diagnostic rating of 'certain' (10 patients), 'probable' (5) and 'possible' (6) was assigned on the basis of clinical criteria. DHPLC mutation analysis of the *COH1* gene is ongoing. Until now, we have analyzed 20 exons out of 62, identifying mutations in four patients: three point mutations in genetic compound state and one homozygous intragenic deletion spanning from exon 6 to exon 16. In addition, mutation analysis revealed three intronic variants of unknown significance (IVS 2-60 A>G, IVS 17+61 C>A, IVS 21-86 A>T). At present, mutations in the *COH1* gene were identified in patients clinically classified as 'certain' and 'probable', while no mutations were not found in patients classified as 'possible'.

P0661. An inhibitory sequence in human Collagen XVIII promoter modulates its transcription in hepatocytes

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Collagen XVIII is an heparan sulfate proteoglycan constituent of liver basal membrane. Proteolytic cleavage of its c-terminal region originates endostatin, a potent angiogenesis inhibitor. Angiogenesis plays a crucial role in hepatocellular carcinoma (HCC) progression.

Reduced Collagen XVIII expression is associated with larger and higher vascularized HCC tumors and, lower endostatin serum levels in the blood of HCC patients have been associated with larger tumors. Elucidating the factors that control Collagen XVIII expression in hepatocytes is important to understand its role in HCC progression. We recently showed that Sp1, Sp3 and YY1 bind human Collagen XVIII promoter controlling liver expression. We characterized a SNP in this region, -700 T/G, which influences transcription level. Allele G, most common among African descendents (55%), has higher (39%) transcription activity than allele T, most common among European descendents (58%). Sp3 has higher affinity for the G allele, while YY1 has higher affinity for the T allele (Armelin-Correa et al., Matrix Biol. 2005; 24:550-9). Sp3 and YY1 can activate or inhibite transcription in different contexts. Deletion of a sequence of 40bp containing the SNP-700 caused 31% increase in promoter activity when compared with the promoter containing the T allele. These results suggest that these 40bp contain an inhibitory element. We are now performing cotransfection experiments to elucidate the role of Sp3 and YY1 in Collagen XVIII expression in hepatocytes and, to verify if the different interactions of YY1 and Sp3 with both alleles of SNP-700 are responsible for their difference in transcriptional activity. FAPESP/CEPID,CNPq

P0662. Sonic Hedgehog Dependent Proliferation In A Series Of Human Colorectal Cancer Patients

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Background : The Hedgehog (Hh) gene family activation, known to regulate stem cells, is responsible for the induction of GLI1 protooncogene and subsequent cellular proliferation. Sonic Hedgehog (SHh), one of the Hh family members promotes. SHh is also expressed in colonic stem cells at the base of the colon villi. As differentiated colonic cells arose from the constant renewal of Hedgehog-expressing colonic stem cells, SHh could be involved in human colonic carcinogenesis.

Methods: Tissue-samples of colorectal adenocarcinoma (T) and adjacent normal colon tissue (NT) were drawn for each of 44 consecutive colorectal cancer patients. Transcription of SHh, GLI1 and FOXM1 were quantified using RT-PCR. Similar mRNA in vitro measurements of GLI1 and FOXM1 after specific induction by SHh-Np were performed in the HT-29 colorectal tumor cell line.

Results: SHh was overexpressed in colorectal adenocarcinomas of 86% patients. In vivo GLI1 and FOXM1 transcription levels were correlated with SHh induction (SHh vs. GLI1 r=0.77*, GLI1 vs. FOXM1, r=0.68*, SHh vs. FOXM1, r=0.79*, *p<0.0001). In parallel, in vitro SHh induction of HT-29 colorectal cell line induces cell proliferation supported by GLI1 then FOXM1 mRNA production. SHh overexpression did not correlate with the patient characteristics evaluated.

Conclusions: We demonstrated SHh activation in human colonic adenocarcinomas and in a colorectal cell line with subsequent GLI1 and FOXM1 activation known to promote cell proliferation. This

activation within human colorectal adenocarcinoma tissue confirms the proliferating role of the Hh pathway in colorectal carcinogenesis and suggests a potential therapeutic target of Hh blockade in colorectal cancer.

P0663. The prevalence of Connexin 26 (*GJB2*) gene mutations in Turkey

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Congenital sensorineural hearing loss (deafness) is a complex disorder that involves a high number of genes and environmental factors, which affects approximately 1/1,000 live births. Recently, there has been enormous progress in non-syndromic deafness research with the identification of over 50 loci and 15 genes. Among these, GJB1, GJB2, GJB3, and GJB6, encode for connexin proteins as Connexin32, Connexin26, Connexin31, and Connexin30, respectively. Mutations in these genes cause autosomal recessive, autosomal dominant or X-linked hearing impairment, both syndromic and non-syndromic. The mutations in *GJB2* gene have been described as a major cause of congenital deafness and account for about 50% of all congenital cases.

The aim of this study was to determine the prevalence of GJB2 gene mutations in Turkey. We have studied 100 affected cases with prelingual severe-to-profound deafness. The entire coding region of the *GJB2* was directly sequenced in all patients for detection of *GJB2* mutations. Of these 100 cases, 30 cases (30%) had at least one mutant *GJB2* allele. Among these, twenty cases were homozygous for 35delG mutation, three cases were heterozygotes for 35delG mutation, four cases were heterozygotes for D50N mutation, one case was homozygous for P173S mutation, one case was compound heterozygotes for 35delG/delE120/K122I and one case was compound heterozygotes for 35delG/312del14bp mutation. Besides, multiple polymorphisms such as E42E, E114G, G12G, G160S, V27I were also detected.

In conclusion, the high prevalence of mutations in *GJB2* in some populations provides the tools for molecular diagnosis, carrier detection, counselling and prenatal diagnosis of congenital hearing impairment.

P0664. Screening of 1q41-q42.12 and 15q26.1-q26.2 regions by multiplex ligation-dependent probe amplification (MLPA) in patients with congenital diaphragmatic hernia (CDH)

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Congenital Diaphragmatic Hernia (CDH) is characterized by incomplete formation/muscularization of the diaphragm, often accompanied by lung hypoplasia and pulmonary hypertension. It has an incidence of ~1/3000 live births. In most cases the etiology is unknown.

In our ongoing study entitled "Gene Mutations and Rescue in Human Diaphragmatic Hernia", we have recruited over 173 patients. Ninety-nine cases were classified as having isolated CDH, while the remainder had complex CDH.

Our aCGH and FISH studies demonstrated a *de novo* 1q41-q42.12 deletion in a Fryns syndrome [OMIM # 229850] patient with CDH. Another Fryns -like patient with CDH had a cytogenetically visible deletion overlapping this region where several different chromosome abnormalities have been previously reported in CDH patients. We suggest that deletion or disruption of gene(s) in this locus can lead to CDH, Fryns syndrome, or a Fryns syndrome phenocopy. A 5 Mb chromosome 15q26.1-q26.2 deletion interval containing four known genes has recently been reported as another potential critical CDH region.

Using Multiplex ligation-dependent probe amplification (MLPA) (MRC-Holland, E2 kit) we have confirmed, and more precisely delineated the 1q41-q42.12 deletion interval to a 6.5 Mb region containing 29 known genes. We are currently screening all our CDH probands for 1q41-q42.12 and 15q26.1-q26.2 microdeletions or microduplications using customized MLPA synthetic probes, as this assay is efficient and reliable for dosage screening of multiple loci in a single reaction.

P0665. Identification of mutations causing congenital muscular dystrophies

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The congenital muscular dystrophies (CMD) are a heterogeneous group of autosomal recessive disorders. It is often difficult to establish a definite clinical diagnosis and offer prenatal diagnosis without identifying the causative mutations in one of a number of possible candidates.

We currently offer a mutation screening service for the LAMA2, FKRP and SEPN1 genes. Causative mutations have been identified in 20/79 individuals screened for FKRP mutations, 13/77 for SEPN1 mutations and we have identified LAMA2 mutations in 18/45 cases, but analysis is incomplete for the 27 cases without identified mutations. Four patients with FKRP mutations were originally referred with a diagnosis of Becker muscular dystrophy, demonstrating the clinical overlap with other muscular dystrophies. Linkage analysis combined with immunohistochemical analysis has enabled us to carry out 9 prenatal diagnoses for MDC1A in the absence of characterised LAMA2 mutations.

A subset of CMDs (including Walker-Warburg syndrome, Muscle-Eye-Brain Disease and Fukuyama CMD) are associated with mutations in genes coding for proteins that are putative or demonstrated glycosyltransferases. We have set up a protocol to simultaneously screen for mutations in five of theses genes; POMT1, POMT2, POMGnT1, Large and Fukutin, in 100 patients to ascertain frequency and severity spectrum of these conditions. We are using multiplexed PCR and heteroduplex analysis using Temperature Gradient Capillary Electrophoresis (TGCE) on a Reveal Discovery System (SpectruMedix) which uses ethidium bromide detection of heteroduplex PCR fragments. This approach is also used to screen LAMA2 whilst the smaller genes such as FKRP and SEPN1 are screened by direct sequence analysis.

P0666. *CHRND* mutation causes a congenital myasthenic syndrome by impairing coclustering of the acetylcholine receptor with rapsyn

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Mutations in various genes encoding proteins expressed at the neuromuscular junction may cause a congenital myasthenic syndrome (CMS). Mutations of acetylcholine receptor (AChR) subunit genes lead to endplate AChR deficiency or to altered kinetic properties of the receptor. Mutations in the alpha, beta and delta subunits of the AChR are less frequent than mutations of the epsilon subunit and often associated with a severe phenotype.

In a sporadic CMS patient of German origin, we identified two compound heterozygous mutations in the *CHRND* gene encoding the delta subunit of the AChR: a novel point mutation in the long cytoplasmic loop, *CHRND* E381K, and a 2.2 kb microdeletion disrupting the *CHRND* gene. As the cytoplasmic loop of the AChR subunits is known to be essential for AChR-rapsyn coclustering, we studied the interaction of AChR containing the *CHRND* E381K mutation with rapsyn. Interestingly, the mutated receptor showed severely reduced cluster formation compared to the wildtype receptor. By contrast, the corresponding amino acid substitution in the cytoplasmic loop of the AChR epsilon (*CHRNE* E376K) as well as a recently reported CMS mutation affecting this domain (*CHRNE* N436del) had no impact on cluster formation.

Conclusion*: CHRND* mutations are a rare cause for CMS but should be considered in patients with a severe, early-onset disease form, with recurrent episodic apneas. Our results suggest that impairment of AChR-rapsyn coclustering - a well-known molecular mechanism

for rapsyn mutations - could also result from mutations in the AChR subunits.

P0667. A large deletion involving the connexin 26 gene

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Mutations in the *GJB2* gene coding connexin 26 are the most frequent cause of congenital non-syndromic hearing impairment (NSHI) in Caucasian populations. Recently two large deletions involving the connexin 30 gene were described in NSHI patients with a GJB2 mutation in trans. We report here for the first time a deletion of *GJB2* in a patient with congenital, profound hearing impairment associated with developmental delay.

Screening for *GJB2* mutations was carried out by denaturing high-performance liquid chromatography (DHPLC) followed by sequencing. The patient appeared to be homozygous for a new *GJB2* mutation c.250G>A, p.Val84Met. Known *GJB6* deletions analyzed by specific PCR were absent and no mutation was founded in the first non-coding exon of *GJB2.* The mutation p.Val84Met is potentially deleterious because Valine 84 is evolutionarily highly conserved and was not observed in 200 chromosomes of normal-hearing individuals. The mutation was heterozygous in the patient's father, but absent in the mother and in the maternal grand parents. Uniparental disomy was excluded by genotyping with a set of 8 markers from the long arm of chromosome 13. A set of 6 microsatellite markers in 13q12-11 analysed in the family confirmed a large deletion involving *GJA3* (CX46), *GJB2* (CX26) and *GJB6* (CX30) in the patient and his mother. Our results indicated that the patient was compound heterozygous for [pVal84Met]+[del(*GJA3-GJB2-GJB6*)] and that this genotype can induce deafness. This observation shows the importance of *GJB2* analysis patients with hearing impairment and the segregation study of the GJB2 mutations.

P0668. GJB2 gene analysis in non-syndromic hearing loss in a sample of Mexican patients

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Non-syndromic hearing loss (NSHL) can be caused by mutations in any one of a large number of genes. Half of severe childhood deafness is due to mendelian inheritance, about 87% of this group presents an autosomal recessive inherited pattern. Mutations in the GJB2 gene, encoding the connexin 26 gap-junction protein, account for a significant proportion of NSHL. Different population distributions of more than 70 GJB2 gene defects have been described in the literature. The aim of the present study was to investigate the prevalence of mutations in the GJB2 gene, especially 35delG mutation, in patients with NSHL in a sample of Mexican population. The study was approved by the Ethics Committee of the General Hospital of Mexico. We ascertained 8 unrelated families with NSHL showing an apparently autosomal recessive pattern. Informed consent was obtained from all participants and their parents. Audiometry was performed in all patients. DNA was extracted from peripheral blood while GJB2 gene analysis was performed in ABI PRISM 310 genetic analyzer. GJB2 mutations were detected only in one family, this mutation was present as heterozygous state. This study revealed that Mexican population presents a low prevalence of GJB2 sequence variations causatives of NSHL.

P0669. Depletion of copper transporting gene transcription in rat brain with experimental dementia of Alzheimer type (EDAT)

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Present-day conception of neurodegenerative disease pathogenesis suggests that leading role in the process belongs to disturbance of copper metabolism. The expression of genes coding for copper transport proteins was studied in hypothalamus, hippocampus and amygdala in Wister line's rat with EDAT resulting from the administration in fourth ventricle of cerebrum 15 aminoacid peptide corresponding to β-amyloid from ^{10}Y to -V^{25}. The copper-transporting proteins are: 1) CTR1, a high affinity copper importer; 2) two Cu-transporting ATPases P1 type: ATP7A and ATP7B, participated in metabolic inclosing of copper in cuproenzymes; 3) soluble ceruloplasmin (Cp) and GPI-anchored Cp; 4) precursor of β-amyloid (APP) and cellular prion (PrPc), having a capacity for a high affinity binding of copper ions and catalyze the reduction Cu(II)→Cu(I) necessary for import via CTR1. The formation of β-amyloid aggregate was detected histologically, development of dementia was checked by the decrease of conditioned reflex making, relative mRNA concentrations were determined by semiquantitative RT-PCR, the Cp content was measured by rocket immunoelectrophoresis, copper concentration was detected by atom-absorption spectrometry. We determined that expression of copper-transporting genes in the brain departments has a region-specific character. Only cells of hypothalamus expressed mRNA of all studied copper-transporting genes. In rat with EDAT, no Cp neither ATPases were found in any brain department. The mRNA of CTR1, APP and PrPc were depleted in several folds. Simultaneously, any abnormalities of copper metabolism in liver and in blood of these rats were not detected. The influence of β-amyloid aggregate on copper metabolism is discussed.

P0670. Father-to-daughter transmission of Cornelia de Lange syndrome caused by a mutation in the 5' untranslated region of the *NIPBL* gene

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Cornelia de Lange syndrome (CdLS) is a developmental disorder characterised by typical facial dysmorphism, growth and mental retardation, microcephaly, behavioural problems, and malformations of the upper extremities. Mutations in the *NIPBL* gene were recently found to cause CdLS. *NIPBL* anomalies are detected in ~40% of reported cases, suggesting genetic and/or further allelic heterogeneity in CdLS, some mutations being not detected by current screening methods. To test whether mutations in the 5' untranslated region (5'UTR) and proximal promoter of the *NIPBL* gene could contribute to the pathogenesis of CdLS, we screened a cohort of 21 CdLS patients with no previously identified mutation by direct sequencing of this part of the gene.

This work allowed the identification of a small heterozygous deletion-insertion mutation in exon 1, 321 nucleotides upstream of the translation initiation codon, in one affected girl and in her mildly affected father. This mutation, which altered a nucleotide that is highly conserved across species, occurred *de novo* in the father. Moreover, it was not detected in 388 control alleles. Using real-time quantitative PCR, we showed that *NIPBL* mRNA expression was lowered in both patients' lymphocytes compared to control samples.

Our results demonstrate that mutations in the 5' non coding region of the *NIPBL* gene may be involved in the pathogenesis of CdLS. Moreover, the mutation reported here expands the spectrum of *NIPBL* anomalies in CdLS and suggests that mutations affecting the regulatory or untranslated region of the gene might be associated with a mild phenotype.

P0671. Mutations causing craniosynostosis, cleidocranial dysplasia and parietal foramina detected by the craniofacial molecular service in Oxford, UK

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The craniofacial service was set up in Oxford, UK in 2002 to provide

molecular testing of the *FGFR1*, *FGFR2*, *FGFR3* and *TWIST1* genes in patients with syndromic craniosynostosis and their relatives. In 2004 this service was extended to provide molecular testing of the *RUNX2* gene in cases with cleidocranial dysplasia and of the *MSX2* and *ALX4* genes in patients with parietal foramina. Most recently a molecular testing service for the *EFNB1* gene in cases with X-linked craniofrontonasal syndrome has been set up, and a new craniofacial MLPA kit developed to test for deletions and duplications of all these genes.
Since 2002 we have analysed 214 new cases with craniosynostosis and identified pathogenic changes in 28.5%. Familial testing has been carried out in 102 relatives with 33.3% found to carry the familial mutation. Of the mutations identified, 7 are novel - 3 in *TWIST1*, 3 in *FGFR2*, and 1 duplication of *RUNX2* detected by MLPA and confirmed cytogenetically. Brief case histories of 2 of the novel *FGFR2* mutations (p.E565A and p.N549T) and the *RUNX2* duplication will be presented.
Since 2004 we have also identified mutations in 13 out of 16 cases (81.3%) with cleidocranial dysplasia and 1/3 cases (33.3%) with parietal foramina. A brief summary of the mutations identified will be presented.

P0672. Identification of a novel missense mutation in the CRYGD gene causative of nuclear congenital cataract

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Cataract is the leading cause of reversible blindness in childhood with an occurrence of 1-6/10,000 live new born. Cataracts are characterized by the location and structure of opacities, i.e. shape, size, color and refractive quality. Cataract may be an isolated anomaly or part of a syndrome. The majority of inherited non-syndromic cataracts are transmitted as an autosomal dominant trait. Mutations in the CRYG genes, which encode the main cytoplasmic proteins of the human lens, have been associated with cataracts of various appearances. The aim of the present study was to identify the disease locus for nuclear congenital cataract in a non-consanguineous family with two affected members. DNA from leukocytes and bucal swab was isolated to analyze the CRYGA-D cluster genes and to discard paternity through gene scan with several highly polymorphic markers. DNA sequencing analysis of the two affected members showed a novel heterozygous missense mutation in the CRYGD gene. Analysis of the two unaffected members of the family and the normal parents showed a normal sequence of the CRYGA-D cluster genes. Analysis of highly polymorphic markers shows no evidence for non-paternity. This mutation was not found in a group of 120 unrelated controls. In this study we describe a novel mutation in the CRYGD causative of nuclear congenital cataract. Besides, our data strongly suggest that the origin of the mutation was in the germinal line of one of the parents.

P0673. Novel large rearrangement in the CFTR gene in cystic fibrosis patients from Reunion Island

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The Reunion Island (RI) is a French province, 800 kms to the east of Madagascar, and 200 kms to the west of Mauritius. In RI, the birth prevalence of the Cystic fibrosis (CF) is particularly high in the population of European origin. In a previous study we have demonstrated that the screening of the 27 exons of the CF transmembrane conductance regulator (CFTR) gene by DHPLC allowed the detection of 93% of the molecular defects present in RI. Unidentified CF mutations may lie in introns or in regulatory regions, or correspond to gene rearrangements at the heterozygous state which escape detection using current PCR based techniques. Using a combination of different methods, 6 of the 13 unidentified CF alleles were found to harbor a novel deletion of 5288 bp, spanning the exons 17a, 17b and 18. This accounts for 46% of unidentified alleles. Identification and examination of the breakpoint sequences showed that this deletion is different from the 3120+1Kbdel8.6Kb previously found in the Palestinian arabs. The IVS16+3316_IVS18+644del5288 bearing chromosomes had a common intragenic haplotype. This mutation causes an in frame deletion of 160 amino acids that are part of the transmembrane domain 2 of the CFTR protein. Clinical evaluation of homozygotes (n=2) and compound heterozygotes (n=2) indicate that this deletion represents a severe mutation associated with positive sweat test, pancreatic insufficiency and early age at diagnosis. In conclusion, we have shown that this novel gross genomic rearrangement is the fourth most common mutation in CF patients from RI.

P0674. Cystic fibrosis diagnostics in Kaunas Medical University Hospital (Lithuania)

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Cystic Fibrosis (CF) is one of the most common inherited disease in European population. 2004 - 2005 years 22 patients suspected with CF diagnosis were tested in Kaunas Medical University Hospital. We analysed patients DNA from blood samples for simultaneous detection and identification of the commonest 19 Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene mutations in European population (with INNO - LiPA CFTR 19 kit, INNOGENETICS, Belgium).
Results: 3 patients (13,6 %) had genotype F508del/F508del. 5 patients were heterozygotes for mutation F508del (genotype F508/N; N - normal). 14 patients had neither F508del nor another mutations from the 19 tested mutations.**Conclusion:** The frequency of F508del/F508del homozygotes (13,6 %) is lower than in Western European CF patient's groups. The explanations of results could be different frequency or (and) another mutated alleles in Central and Eastern, and Western European population or incorrect clinical suspicion of Cystic Fibrosis

P0675. Molecular genetic analysis of SLC3A1 and SLC7A9 genes in Greek cystinuric patients

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Cystinuria is one of the most common inheritable disorders of amino acid transport with an incidence of 1:7000. It is caused by the defective transport of cystine and three other dibasic amino acids in the brush border membrane of proximal venal tubules and intestinal track. It is characterized by the development of cystine stones. Up to date two genes are mainly involved in cystinuria, SLC3A1 and SLC7A9. The aim was to identify the mutations in these two genes in a cohort of Greek patients. We obtained DNA form 18 Greek patients and 7 first-degree relatives. All patients had formed cystine stones and were subjected in surgery. With PCR and DGGE analysis we found four different mutations in the SLC3A1 including one novel one, 2 missense mutations (T216M, M467T), and one duplication (Duplication exon 5 - 9). In the SLC7A9 gene we found 2 missense mutations (S379R G105R), one deletion (c1388delA) and four polymorphisms (1143 C/C, c399 C/T, c411 T/C, 1365 C/T). The results were confirmed by sequencing analysis. The most frequent mutations found were M467T and T216M. Moreover 15 patients were diagnosed as compound heterozygotes, which shows the molecular heterogeneity of the disease. The distribution of mutations that cause cystinuria in Greek patients can contribute to the estimation of the frequency of the disease in Greece. In addition, it will enable genotype-phenotype correlations, which can lead to career identification and targeted prenatal diagnosis.

P0676. Mutation screening of the basal promoter and of exon 1 of *GJB2* in hearing impaired patients with monoallelic mutations in exon 2

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GJB2 related deafness is mostly due to recessive mutations. However, mutation screening of *GJB2* has revealed a significant percentage of hearing impaired patients with only one recessive *GJB2* disease-causing mutation, or one mutation with uncertain pathogenic significance, which could not clearly account for the hearing loss by its own. Most of those studies performed to date only involved the analysis of the *GJB2* coding region. Therefore, mutations in non-coding regions may have been missed. In fact, there are no sequence variants described in the exon 1, and there are only a few polymorphisms reported in the promoter.

At least four mutations in non-coding regions of *GJB1* gene have already been described in patients affected by X-linked dominant Charcot-Marie-Tooth neuropathy, three of which have been found in the nerve-specific promoter and the fourth one in the 5' UTR of the mRNA. Taking into account these findings in *GJB1*, the existence of disease-related mutations in the promoter or in the exon 1 of *GJB2* gene appears to be likely.

In order to investigate that possibility, in the present study we have analysed the *GJB2* basal promoter and exon 1 of Portuguese hearing impaired patients who presented with only one pathogenic or uncertain mutation in *GJB2* coding region.

Within our results, we present a substitution (-3224C>A) in exon 1 of a normal hearing control subject, which is, to our knowledge, the first sequence variant of *GJB2* exon 1 described to date.

P0677. Spectrum and carrier frequency of *GJB2* (Cx26) mutations associated to deafness in Portuguese families

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Many disease-causing mutations have been described in the *GJB2* gene, and several populations have recurrent mutations, such as the 35delG among Caucasians, 235delC in the Japanese, and 167delT mutation in the Ashkenazi Jews. Because of the high frequency of *GJB2* mutations in most populations, mutation analysis of this gene is widely available as a genetic diagnostic test. However, the mutation spectrum in *GJB2* can diverge substantially between populations. Therefore, studies are needed to determine the contribution of *GJB2* variants in different populations.

In a previous study we have investigated a dataset from Portuguese families with non-syndromic, bilateral, sensorineural hearing loss, and biallelic mutations in *GJB2*, in order to estimate the prevalence of these variants in the Portuguese impaired population, and to assess variability among subjects enabling to establish a possible genotype-phenotype correlation.

In the present study we have investigated all the Portuguese affected families previously screened for *GJB2*, in which we have identified at least one mutation in this gene. The aim of the study was to determine the spectrum of *GJB2* variants identified so far and to estimate its carrier frequency in a sample of about 100 unrelated, normal hearing individuals. Estimation of 35delG carrier frequency had already been performed in our lab, involving a larger control sample.

The molecular methodologies have included DNA extraction and amplification, SSCP, enzymatic restriction, allele-specific PCR and automated sequencing.

* A.R. and C.T. contributed equally to this work

P0678. Prevalence of the del(*GJB6*-D13S1830) mutation in the *DFNB1* locus in Portuguese patients with non-syndromic hearing loss

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A deletion not affecting *GJB2* but truncating the neighbouring *GJB6* gene, (encoding connexin 30), was identified in 2001, and hypothesized to also contribute to *DFNB1* related hearing loss. Effectively, this deletion, later called del(*GJB6*-D13S1830), was shown to be the accompanying mutation in many cases in which affected subjects were *GJB2* heterozygotes, thus only carrying one mutant allele.

These findings led many research groups all over the world to screen their patients with non-syndromic prelingual deafness, and carrying one or null *GJB2* mutant allele, for the presence of del(*GJB6*-D13S1830) The results obtained, namely in a large multicenter study, involving patients from nine countries, revealed that the *GJB6* deletion is present in most of the screened populations, but with quite different frequencies.

In a preliminary study previously performed in our laboratory, the deletion was absent in the 74 unrelated patients then tested (0/148 chromosomes), in a clear contrast with the high prevalence observed in Spain, but in accordance with its scarcity in Italy.

In order to better elucidate these differences and establish a precise picture of the Mediterranean countries, the aim of the present work was thus testing a larger Portuguese sample for del(*GJB6*-D13S1830) mutation.

On the total, we have screened over 150 unrelated patients with non-syndromic hearing loss, heterozygous for a recessive *GJB2* mutation or with null mutation in *GJB2*, using Multiplex PCR.

None of the individuals was a carrier of the del(*GJB6*-D13S1830) mutation, which indicates that this mutation, if at all present, is very rare in the Portuguese deaf population.

P0679. Incidence of GJB2 mutations in the Greek population.

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About one in 1000 children is affected by prelingual deafness. In developed countries at least 50% of the cases is due to genetic defects. Among genetic deafness, most frequent are the non-syndromic forms (70%), of which autosomal recessive inheritance predominates (80%). More than 40 DFNB loci for non-syndromic autosomal recessive deafness have been mapped to human chromosomes and 23 of the genes have been identified to date. The major recessive locus is DFNB1 with the responsible gene GJB2 encoding connexin 26 and with one frameshift mutation (35delG) responsible for the majority (until 85%) of GJB2 deafness mutations in Caucasians. We have previously determined a carrier frequency of the 35delG mutation of 3.5% in the healthy Greek population and found biallelic GJB2 mutations in 33.3% of Greek children with non-syndromic prelingual deafness. Other GJB2 mutations than 35delG seem to have different geographical distributions. In the present study we determined the frequency of four already identified recurrent mutations in the Greek population (W24X, L90P, delE120, and R184P). Easy screening techniques for the four different mutations (ARMS-PCR and PCR-RFLP) were developed. The material consisted of 200 unrelated hearing Greek controls and 26 Greek sib pairs with non-syndromic, prelingual deafness, tested negative for 35delG. The L90P mutation was found in 4/200 controls and might be frequent in the Greek population, as previously found in Austrian deafness patients.

P0680. The *GJB2* R75Q (c.224G>A) mutation is associated with variable phenotype and spread among different genetic backgrounds.

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The *GJB2* mutations, the most common cause of autosomal recessive hearing loss, are also involved in rare cases of autosomal dominant deafness and/or deafness with dermatological features. The dominant mutation R75Q (c.224G>A) has been so far identified in several families:

one Turkish case, two French families, and a family of mixed origin from Siberia. These published cases show that variable hearing loss is observed and is associated with variable manifestation or absence of skin alteration. We described a multiplex family with dominant and pseudo-dominant deafness resulted from combination of different *GJB2* mutations. Now we focus on this family branch where c.224G>A was first found as the sole mutation or in *trans* of the recessive *GJB2* mutations. Nevertheless, all affected family members carrying the c.224G>A mutation (grandfather, son, daughter, and two grandsons) present a uniform deafness phenotype: prelingual severe-profound hearing loss. Documented dermatological examination did not reveal any skin disorder in the grandfather and his daughter both genotyped [c.224G>A]+[=] nor in his son with the [c.224G>A]+[c.235delC] genotype, though mild keratosis manifestation was detected on the grandsons' legs both genotyped [c.224G>A]+[c.313_326del]. Because of variable penetrance regarding skin disorder symptoms, the c.224G>A status (nonsyndromic and/or syndromic) remains ambiguous. Our data suggest that the phenotypic variability of hearing loss caused by c.224G>A observed among other affected families may rather reflect the effect of modifier genes and/or environmental factors than the c.224G>A genetic background.

P0681. Detection of mtDNA mutations in 12S rRNA gene (MTRNR1) and tRNA-Ser(UNC) gene (MTTS1) using DHPLC in patients with nonsyndromic hereditary hearing impairment

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Since 2001 we have analysed 601 subjects, affected by neurosensorial deafness with various degrees of hearing loss. Mutations in the GJB2 gene, encoding for the gap-junction protein Connexin26 (Cx26), are responsible for the majority of non-syndromic recessive deafness and among these the 35delG allele is the most common in the Mediterranean population. In our cohort of patients we identified 169 subjects affected by Cx26 and/or delta(GJB6-D13S1830) deafness; among these 102 (60,3%) exhibited a 35delG homozygous genotype, 47 (27,8%) were compound heterozygous 35delG/not-35delG while16 patients (9,5%) were compound heterozygous not-35delG and 4 showed a dominant mutation.

Mutations in MTRNR1 gene (A1555G, 961delT), encoding 12S ribosomal RNA, account for most of the cases of hereditary deafness induced by amino glycoside's administration. We screened all the affected patients, with one or without Cx26 recessive mutations, for the A1555G substitution by DHPLC, and the positive samples were subjected to sequencing analysis. We found 5 patients carrying the A1555G and the subsequent family analysis led to the identification of this mutation in 6 relatives (4 of these were pre-symptomatic). Mutations in the MTTS1 gene, encoding the Serine tRNA, are an additional cause for nonsyndromic maternally inherited hearing impairment with onset in childhood.

Until now at least 4 MTTS1 mutations have been described (A7445G, 7472insC, T7510C, T7511C). We will discuss the results of the analysis of this hot spot region in our 421 unresolved cases using the DHPLC screening method in order to investigate the prevalence of these mutations in our population.

P0682. Autosomal recessive, non-syndromic hearing loss in India: a different story?

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Autosomal recessive inheritance contributes to 75-80% of prelingual, bilateral non-syndromic hearing loss of genetic etiology (DFNB) and about 50% of these cases are attributed to homozygous mutations in GJB2, worldwide. Although hearing impairment is an important health problem and consanguineous marriages are relatively common, data on the genetics of hearing loss in India are largely inadequate to provide genetic testing for carrier screening or prenatal diagnosis.

In order to find out the prevalence of GJB2 mutations in Indian population, we analyzed DNA samples from 220 individuals who have congenital, bilateral, severe-profound, sensorineural non-syndromic hearing loss and family history consistent with DFNB. Contrary to the major contribution from GJB2 mutations, our data revealed that homozygous or compound heterozygous GJB2 mutations contributed for only 19% (42/220) of DFNB. Heterozygous GJB2 mutations were detected in 21% (47/220). The common mutations included W24X, R127H, W77X while M1V, V27I, 35insG were only occasionally found. Sequencing of the non-coding exon 1of GJB2 did not reveal any pathological variation in families analyzed to date. Currently, we are analyzing GJB6 gene both for the common 342 kb deletion or other mutations and plan to perform linkage analysis for all the known loci for DFNB.

There is a need to expand the search for other DFNB genes in India in order to find out the major players apart from GJB2 and offer a panel of common mutations for genetic testing and primary prevention of NSHL. The results of our ongoing study will be discussed in the current paper.

P0683. Study of Dystrophin gene's hotspots in 23 Iranian families suspected to DMD or BMD

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The dystrophinopathies_Duchenne muscular dystrophy (DMD)and Becker muscular dystrophy (BMD)_are the most common inherited disorders of muscle. Although reliable prevalence data are lacking, the prevalence of DMD is generally estimated at 1:3,500 live male births . Both DMD and BMD are due to mutations in the dystrophin gene , located at Xp21,which comprises 79 exons and 8 tissue-specificpromoters distributed across ~2.2 Mb of genomic sequence_ making dystrophin the largest gene yet described.

Dystrophin gene deletions are found in ~55%of patients with BMD and 65% of patients DMD; point mutations account for ~30% of mutations, and duplications account for the remainder.

Genetic testing for deletions relies on a multiplex PCR technique, with amplification of fragments containing 20 of the gene's 79 exons and with deletions detected as absent or size-shifted bands on poly Acryl amide gel analysis. Because deletions tend to occur in "hotspots" within the dystrophin gene, analysis of this limited number of exons can detect 98% of dystrophin deletions.

Hot spots are exons3-19 and 42-60.We studied all of these exons for 23 Iranian families.

In our study most common of deletion were in exon6, exon44, exon50, exon4 respectively.

P0684. Modifications of chromosome 21 gene expression levels in lymphoblastoid cell lines from Down syndrome patients

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Down syndrome (DS) is the most common genetic cause of mental retardation in human. Although chromosomal abnormality has been characterised by Lejeune in 1959, molecular mechanisms by which total or partial triplication of chromosome 21 (HSA21) leads to perturbation of the phenotype remain unknown. The elementary hypothesis about triplicated gene expression is that they are 1.5 fold overexpressed.

We have chosen microarray technology to explore HSA21 gene expression in a cellular model of DS. A specific HSA21 microarray containing 671 aminomodified oligonucleotides representing 275 genes and orf, 119 predictions and 21 antisense transcripts was designed using appropriate softwares (Golfier *et al*) and spotted on CodeLink® glass slides.

HSA21 gene expression in lymphoblastoid cell lines established from 10 patients (DS) and 11 controls (2N) was compared on the HSA21 oligoarray after control of genomic stability of each cell line by

karyotyping.
Cell lines were analyzed using an experimental design which optimizes to 40 the number of differential hybridizations between DS and 2N samples. The statistical modeling of HSA21 gene expression takes into consideration three features: genotype, gender and biological variability. Data were normalized using non HSA21 genes for which expression levels are not affected by DS.
Among the 90 HSA21 sequences that were sufficiently expressed in cell lines, differential analysis revealed that 46 genes were overexpressed with DS/2N ratios from 1.07 to 1.73. More surprisingly, 3 HSA21 genes were significantly classified as invariants. Finally this analysis showed that biological variability has a significant effect on the expression of 21 HSA21 genes.

P0685. Duplications in the DMD gene in DMD/BMD patients in Serbia and Montenegro

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Although duplication mutations in the DMD gene were reported to be relatively frequent early on, the considerable effort to detect them made them largely neglected. This situation has changed only recently with the development of MAPH and MLPA, two easy and versatile methods for the detection of both deletions and duplications.We present here a retrospective study of 123 unrelated DMD/BMD patients (already screened for deletions in the hot spot regions using modified multiplex PCR kits).Rescreening these with MLPA revealed nine duplications, dispersed over the whole gene. This corresponds to a duplication rate of 17% among deletion-negative and 7% among all patients, an ocerall duplication frequency comparable with what previous studies had suggested.The majority of cases contain a simple contiguous duplication, but we also detected one non-contiguous duplication/ triplication. In most non-contiguous duplications reported thus far the 3' end of the gene is affected. Whilst potentially disturbing the reading frame of the mRNA, these mutations would go undetected using standard multiplex PCR screening. These findings emphasize the importance of screening the entire gene for rearrangements and that duplications, compared to deletions, need to be treated with special care.

P0686. Autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD-AD) in a Bulgarian family: case report

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Laminopathies are clinically extremely heterogeneous group of inherited disorders, caused by mutations in the same gene - lamin A/C (LMNA) gene. Lamins A and C are nuclear envelope proteins, representing alternatively spliced forms of the LMNA gene. It was identified that mutations in the LMNA gene cause inherited neuromuscular disorders and/or cardiac conduction disturbances, lipodystrophies, peripheral neuropathy and progeroid syndromes.
Here we report a Bulgarian family with EDMD-AD, caused by a unique mutation in the LMNA gene. Four family members (the index patient, his brother and his two daughters) were tested for mutations in the LMNA gene. The mutation p.Asn195Asp in exon 3 was detected in three of them (one of the daughters was not a carrier). On the other hand, pronounced clinical variability was noticed in this family. The index patient showed disease onset at the age 16, while his carrier brother at the age 30 did not demonstrate any symptoms. His carrier daughter demonstrates mild muscle weakness at the age 17. The creatinekinase levels were normal to slightly increased. The progression was slow to the age of 30s and more rapid afterwards. Muscle weakness and atrophy were more evident in biceps brachii. Contractions in elbows and ankles were present. Cardiac involvement was demonstrated as conduction and rhythm disturbances, 1st degree AV block, left bundle branch block Defibrillator was offered.
The pathophysiological mechanism of these tissue-specific laminopathies as a result of mutations in a gene expressed all over, is unclear and all the data on this point is welcome.

P0687. COL5A1 haploinsufficiency in Russian patients with classic Ehlers-Danlos syndrome

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Classic Ehlers-Danlos syndrome (EDS) is a heritable disorder of connective tissue of which the major diagnostic criteria are hyperextensible skin, joint hypermobility and delayed wound healing with atrophic scarring. Mutations in the COL5A1 gene leading to a non-functional allele and resulting in COL5A1 haploinsufficiency have been shown to cause the disorder in approximately one third of patients with classic EDS. Our study included 27 probands with clinical diagnosis of classic EDS. Cultured dermal fibroblasts and blood samples were obtained from all patients, and mRNA and genomic DNA were isolated subsequently. The expression of COL5A1 alleles was analyzed using three polymorphic sites in the COL5A1 coding region - PstI in exon 5, Bsc4I in exon 58 and DpnII in exon 66. The RFLP analysis was performed initially on genomic DNA, to determine the genotype of each patient, and subsequently on cDNA. Of 27 patients, 23 (85%) were heterozygous for one or several polymorphisms in DNA samples. Four of these 23 patients (18%) showed complete or nearly complete loss of expression of one COL5A1 allele in cDNA sample. The relatively low COL5A1 haploinsufficiency detection rate confirms genetic heterogeneity of classic EDS.

P0688. Arginine-to-cysteine substitutions in the pro-alpha-1(I)-collagen chain result in propensity to arterial rupture

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Type I procollagen is a heterotrimer consisting of two pro-alpha-1(I)- and one pro-alpha-2(I)-chains, encoded by the COL1A1 and COL1A2 genes. Mutations in these genes cause Osteogenesis imperfecta (OI) or Ehlers-Danlos syndrome (EDS), arthrochalasis type. The majority of structural mutations in OI are glycine substitutions in the collagen type I triple helix. In contrast, only two arginine-to-cysteine substitutions in type I collagen have been identified, respectively the p.R134C substitution in two patients with classic EDS and the p.R836C substitution in three families with Caffey disease and mild EDS-features.
We refer on three patients with arterial dissection in early adulthood, who harbour an arginine-to-cysteine substitution in the alpha-1(I)-collagen chain. In addition, patient 1 (p.R134C) presented also classic EDS-features including fragile, hyperextensible skin, whereas patients 2 (p.R396C) and 3 (p.R915C) showed osteopenia with mild skin- and joint hypermobility.
SDS-PAGE of dermal fibroblasts showed disulphide-bonded dimeric-alpha-1(I)-collagen chains which were variably secreted into the medium. Experiments to evaluate the effect of these substitutions on collagen type I maturation and stability showed a delay in amino-propeptide-processing but a normal thermal stability. Ultrastructural findings disclosed disrupted collagen fibrils, variable collagen fibril diameter and interfibrillar granulo-filamentous deposits, reflecting disturbed collagen fibrillogenesis and abnormal secretion of mutant collagen type I.
We demonstrate that arginine-to-cysteine substitutions in type I collagen can result in a phenotype with EDS-like-features and propensity to arterial rupture in early adulthood. This has important implications for genetic counselling and clinical follow-up of patients carrying non-glycine substitutions in collagen type I.
S. Symoens and F. Malfait contributed equally to this work.

P0689. Severe factor V deficiency: identification and molecular characterization of three novel splicing mutations

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Severe factor V (FV) deficiency (MIM+227400) is a rare autosomal

recessive hemorrhagic diathesis, characterized by low or unmeasurable levels of FV antigen and coagulant activity (1% or less). This bleeding disorder has a prevalence of about 1 per million and is associated with clinical manifestations ranging from mild to severe. Among rare inherited coagulopathies, it is one of the least characterized from the molecular point of view.
The mutational screening of the FV gene in three FV-deficient patients identified five genetic defects, three hitherto unknown (the splicing mutations IVS21+1G>A, IVS24+1_+4delGTAG, and IVS8+6T>C, this last being the only one found in the homozygous state), and two already described (Arg1002ter and Arg2074His). To demonstrate the pathogenic role of the newly identified splicing defects, transfections of appropriate FV minigene constructs (either wild type or mutant) were carried out in HeLa cells. RT-PCR analysis on mRNA extracted from transfected cells, demonstrated that the IVS8+6T>C transition causes the entire exon 8 to be skipped from the FV mRNA. Conversely, both IVS21+1G>A and IVS24+1_+4delGTAG cause the activation of cryptic donor splice sites, located in exons 21 and 24, respectively. In all cases, the splicing defect results in the generation of a premature stop codon in the FV protein (p.Lys346SerfsX17, p.Gly1952ValfsX2, p.Met2120IlefsX12, mature protein).
In conclusion this study reports the molecular characterization of three novel splicing mutations responsible for FV deficiency, further supporting the allelic heterogeneity of this disease.
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P0690. MEFV mutations in Tunisian patients suffering from Familial Mediterranean Fever

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OBJECTIVES To identify the frequency and distribution of FMF gene (MEFV) mutations in Tunisian patients.
PATIENTS AND METHODS The study was performed in the Genetic Department of Tunis University Hospital. Patients were referred for genetic study, and counseling. A diagnosis of FMF was made according to published criteria. A diagnostic molecular test was performed for the 5 common known mutations (M694V, V726A, M694I, M680I, E148Q) and for A744S, R761H and I692del. The tests performed were PCR restriction-digestion for M694V, V726A, M680I, R761H, E148Q; ARMS for A744S, M694I and PCR-electrophoresis assay for I692del.
RESULTS Of the 139 unrelated patients investigated, 61 (43.88%) had at least one mutation , studied mutations were absent for others. Of those with mutations, 28 were homozygous, 16 were compound heterozygous, 2 had complex alleles, and 17 patients had only 1 identifiable mutation. Of the mutations, M680I , M694V, M694I ,V726A, A744S, R761H , I692DEL and E148Q accounted for 31.48, 26.85, 12.96, 5.55, 2.77, 0.92, 0.92 and 18.51%, respectively. Five of our patients developed chronic renal failure. Homozygotes M680I patients phenotype is less severe than M694V ones.
CONCLUSION, our findings show that MEFV mutations profile in FMF Tunisian patients is different from other Arabs patients. The M680I is the most common mutation which is found to be at least third range in other Arab, non-Ashkenazi Jews, Turks, Armenians populations. There is low frequency of V726A mutation and high frequency of M694Vmutation. This provides important tools for adapting a molecular diagnostic test for our population and further investigation

P0691. Detection of 1115-1118 Del and 3788-3790 Del in Fanconi Anemia

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Fanconi anemia (FA) is a genetically heterogeneous autosomal recessive syndrome associated with chromosomal instability, hypersensitivity to DNA cross linking agents, and predisposition to malignancy. There are 11 different genes for FA.The genes are termed *FANCA* through *FANCJ*.
Clinical manifestations of Fanconi anemia are disorder of skin, Cardiopulmonary system, kidney, head and face and thumb. Complete blood cell count may reveal trilineage pancytopenia or may only show red blood cells that are macrocytic for age. Thrombocytopenia or leukopenia may precede full-blown aplasia.
The specific role of mutations in the FA genes in the pathogenesis of birth defects, bone marrow failure, or oncogenesis is not yet clear
At least 11 genes are involved in the FA pathway. Forty variants are likely to be pathogenic mutations and there are forty-five polymorphism.
Seventeen of these mutations are microdeletions/microinsertions associated with short direct repeats or homonucleotide tracts, a type of mutation thought to be generated by a mechanism of slipped-strand mispairing during DNA.
For molecular diagnosis Sequence Analysis, ARMs-PCR analysis, Restriction analysis is recommended.
When a sequence variant was identified, additional family members as well as normal chromosomes were screened by SSCP and/or restriction analysis to determine if the variant was associated with the FA phenotype.
We investigated these deletions in our patients and we did not find these deletions in non of our patients.

P0692. Molecular characterization of Turkish patients with Fanconi anemia: Preliminary results

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Fanconi anemia (FA) is a clinically and genetically heterogeneous autosomal recessive disorder characterized by bone marrow failure, multiple congenital physical abnormalities, sensitivity to cross-linking agents and predisposition to cancer. At least 11 different complementation groups has been described to date. In Turkey, the most prevalent group is FA-A (~70%), followed by FA-G (14%) and FA-E (10%). The aim of this study is to evaluate Turkish patients belonging to these complementation groups and to identify the underlying pathogenic mutations of the patients belonging to the group A. The subjects of this study were 50 unrelated Turkish families (38 consanguineous). Linkage analysis indicated that 26 families showed homozygosity for the FANCA gene while only 2 and 1 patients were found to show homozygosity for the FANCG and FANCE genes respectively. Mutational analysis of the FANCA gene led to the identification of 3 novel [homozygous C2932T substitution (CAG>TAG) in exon 30 (Q978X); homozygous replacement of T2941C (TGT>CGT) in exon 30 (C281R); ten base-pair deletion between the sequences1360-1370 at the 5’ end of the exon 15], and 3 previously described [homozygous 3639delT in exon 37, heterozygous 3520-3522del in exon 36 (W1174del); homozygous C3263T substitution in exon 33 (S1088F)] mutations in 6 families. The preliminary results of this study indicated that mutation analysis of the FANCA gene is highly difficult; among the patients studied only 6 mutations could be identified, and that mutation spectrum of the gene is highly heterogeneous in Turkish population.
This study was supported by Hacettepe University Research Fund (02G116). <gbalta@hacettepe.edu.tr>

P0693. Polymorphism G664A of atrial natriuretic peptide is associated with lipid levels in familial hypercholesterolemia

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Atrial natriuretic peptide (ANP or NPPA) is the precursor protein of the amyloidosis form called isolated atrial amyloid (IAA) related to the increased incidence of cardiac pathological conditions in ageing. Characteristics of familial Hypercholesterolemia (FH) patients are the high LDL-C levels, which frequently gives rise to premature coronary artery disease (CAD). However, not all FH patients have the same clinical phenotype. The aim of the present study was to assess the relationship between ANP polymorphisms and apolipoprotein (Apo) A1 levels on the likelihood of having CAD in FH patients. The effect of transition T2238C, which leads to ANP with 2 additional arginines and

G664A resulting to a missense mutation Val7Met on lipid values and clinical phenotype were investigated, in 83 FH patients. ApoA1 and HDL-C levels were lower heterozygotes at position 664 compared to GG homozygotes at the same position (121.66±16.06 *vs* 141.38±23.6 mg/dl, p=0.02; 37.02±5.87 vs 48.05±15.41, p=0.05, respectively). ApoA1 concentration remained significantly associated with ANP G664A polymorphism after adjusting for age and sex (p=0.015). No association was found between the G664A polymorphism and CAD in our population. Moreover, ApoA1 and HDL levels were not different among the different genotypes of the T2238C polymorphism, even after adjusting for age and sex. Our results suggest that the A allele at position 664 of the ANP gene is associated with lower levels of ApoA1 and HDL-C in FH patients, but not with CAD risk. Concerning the T2238C polymorphism, no effect could be evidenced neither on lipid parameters nor on CAD incidence.

P0694. Phenotype-genotype correlations in filaminopathies A

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Introduction: Filamin A, encoded by the *FLNA* gene located on chromosome Xq28, is a cytoskeletal protein that cross-links actin in a regulated fashion. Mutations in *FLNA* have been associated with various phenotypes including periventricular heterotopia (PH), oto-palato-digital syndrome type 1 and 2 (OPD), Melnick-Needles syndrome (MNS) and frontometaphyseal dysplasia (FMD). We have analyzed the *FLNA* gene in 48 patients who presented clinical and/or brain MRI signs that belong to the spectrum of filaminopathies A.

Material and Methods: Thirty-two patients had PH, ten had OPD, two had MNS and four had complex congenital malformation disorders. We used the dHPLC technology for an indirect search of mutation in the entire coding region (47 exons), the 5' and 3' UTR regions, and in the intron-exon boundaries. All variants identified using dHPLC were secondary sequenced.

Results: We have identified nine novel different mutations in ten patients with PH. The p.Pro207Leu missense mutation was observed in six members of a large OPD family, and two novel missense mutations (p.N187Ser and p.Ser1199Leu) in two unrelated OPD patients. The previously known p.Ala1188Thr mutation was observed in the two unrelated patients with MNS.

Discussion: We have identified eleven novel mutations in *FLNA*. Our data corroborate previous findings concerning the existence of phenotype-genotype correlations in filaminopathies A. Although PH is largely due to loss-of-function mutations, OPD and MNS are caused by mutations leading to the production of a full length protein and providing a gain-of-function effect. The later mutations clustered within a few regions and some are highly recurrent.

P0695. New polymorphisms in the *filaminB* gene: novel candidates for causing disease?

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Filamins can bind to a great variety of intracellular proteins and play a role in diverse cellular processes like cytoskeleton organisation, membrane stabilisation, anchoring of transmembrane proteins and signalling molecules. Mutations in both *filaminA* and *filaminB* genes are in relationship with serious disorders of the skeletal system; *filaminB* is demonstrated to associate with atelosteogenesis type I and III, Larsen syndrome and spondylocarpotarsal syndrome causing skeletal dismorphias of diverse severity. We sequenced the exons of *filaminB* gene of 59 patients with disorders resembling to known phenotypes and 64 of their healthy relatives. 11 new polymorphisms were revealed, 6 of which were associated with amino acid changes. Most of them occurred also in at least one of the parents therefore are not likely pathogenic. One has *de novo* origin: C3176T (Ala1059Val) change in the exon 21 occurred in a 16-year old patient born with diastematomyelia and myelomeningocele; it was not shown in any of parents. The patient has also shortened upper trunk, double scoliosis and composite developmental disorders of vertebrae. It is possible that the observed nucleotide change may be a novel causative mutation to this kind of disorders of skeletal development.

P0696. MEFV mutations in patients with familial Mediterranean fever from the Aegean Region of Turkey

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Familial Mediterranean fever (FMF) which is mostly frequently present in Mediterranean populations is an autosomal recessive disorder. The mutations in MEFV gene are responsible for the disorder. The MEFV gene is located at 16p13.3 and codes a protein, pyrin/marenostrin. More than 50 mutations have been defined in the MEFV gene.

We have restrospectively evaluated the molecular test results of 300 FMF patients referred to the Molecular Genetics Laboratory of Medical Genetics Department, Faculty of Medicine, Ege University, Izmir/ Turkey in the last two years. Patients had been tested for 12 common mutations in MEFV gene using the strip assay method (Innogenetics, Belgium).

Out of 300 patients tested in the Aegean region in Turkey (600 allels), 151 did not carry any mutations, while 82 patients (27.33%) were either homozygous or compound heterozygous, 66 (22%) carried only one detected mutation, and 1 (0.33%) patient had three mutations.

Allelic frequencies for the four most common muatations in the positive groups were 56% (M694V), 12.75% (V726A), 12.28% (M680I) , respectively. The rest of the allels (7.57%) showed rare mutations which were R761H, P369S, K695R, A744S, F475L.

The frequencies of mutations detected in our group compared to the frequencies reported in the other regions of Turkey showed a two fold increase in V726A mutation frequency. No patient showed a M694I mutation which is sometimes evident in other Mediterranean populations.

P0697. Methylation analysis of the Fmr1 Promoter region in X-Fragile patients

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Fragile X syndrome (FRAXA) is the most common cause known of inherited mental retardation. The hypermethylation of the expanded CGG repeat and of the upstream promoter is associated with gene silencing and the absence of the FMR1 protein.

We have studied four male individuals: two X-Fragile (XF) patients and two normal controls by methylation-specific PCR (MSP) and bisulphite sequencing.

Bisulphite sequencing was performed by sodium bisulphite treatment with CpGenome DNA Modification Kit (Chemicon International) followed by PCR reaction. Purified PCR products were sequenced in an ABI 3100 automated capillary DNA sequencer with primers for the antisense strand.

After bisulphite treatment, all cytosines must be converted to uracil except those that are mehylated (5´ methylcytosine).

This change was complete in controls and XF excluding three specific cytosines at 13518, 13551, 13561 positions (GenBank accession number L29074 antisense strand) that were incompletely transformed to U in XF patients.

These findings suggest the possibility that FMR1 methylated promoter could have targets for methyl specific repressor factors at this sites. This fact would explain the protection of these three cytosines surrounding CpG sites against bisulphite treatment.

These results open the possibility of a coordinated transcription inhibition between methylation and methyl-binding (protein) repressors.
Methylation could therefore inhibit FMR1 transcription not only by recruiting histone deacetylases but also by methyl-CpG-binding repressor proteins that specifically recognize specific methyl-CpG - binding sites.

P0698. Markers of genetic susceptibility to gonadothropin induced ovarian response.

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Assisted reproductive technologies are widely used in treatment of infertility, that's why the problems of ovarian response to gonadothropins GT - induced stimulation are updated. This response is tightly connected with ovarian reserve from one side and from the abilities of the individual to metabolize the exogenous GT. The aim of our study was to investigate the associoation between FMR1, INHα1, NAT2, GSTT1 and GSTM1 genes mutations and response to GT-stimulation in donor oocyte population. We investigated 194 healthy oocytes donors, wich were stimulated for oocyte retrieval with GT. The dosage of GT, and quantity of oocytes were valuated in these patients. It was found, that in women with INHa1 gene mutation 769G→A the quantity of oocytes were significantly decreased in comparison to patients without such mutation. It was also shown, that in patients with "slow" acetylation NAT2 genotype and GSTT1 gene deletion the dosage of GT necessary for stimulation was significantly higher than in patients with "quick" acetylation NAT2 genotype. Moreover in women with FMR1 gene intermediate allels (≥ 40 CGG-repeats) in the second cycles the tendency to increasing of the daily GT-dosage was observed Our data demonstrated that FMR1 and INHα1 genes can be involved in GT reception and biotransformation in turn "slow alleles" and GSTT1 0/0 genotype could modify the ovarian response to exogenous GT.

P0699. Expansion, deletions and nonsense mutations in the polyAlanine-containing transcription factor FOXL2 lead to aggregation

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Mutations of *FOXL2*, a gene encoding a forkhead transcription factor, have been shown to cause the blepharophimosis-ptosis epicanthus inversus syndrome (BPES). This genetic disorder is characterized by eyelid and craniofacial abnormalities and is often associated with premature ovarian failure. Several types of mutations have been found in the open reading frame of *FOXL2*, including nonsense and missense mutations and polyalanine expansions or deletion. Premature stop codons in *FOXL2* have been considered so far as null alleles. However, we demonstrated that such nonsense mutations might lead to the production of N-terminally truncated proteins by re-initiation of translation downstream of the stop codon. Surprisingly, the truncated proteins strongly aggregate in the nucleus, partially localize in the cytoplasm and retain a fraction of the wild-type protein. Furthermore we have studied the effect of the polyalanine length variation. We showed that mutant FOXL2 with an expanded polyAlanine tract forms large aggregates both in the nucleus and the cytoplasm of transfected cells, whereas the wild-type protein localizes in the nucleus in a rather diffuse manner. We have also shown that a complete deletion of the polyAlanine tract of FOXL2 induces a significant intranuclear aggregation, sensitive to the action of chaperones. Our results show that at least three types of mutation in FOXL2 lead to protein aggregation and provide the first demonstration of protein aggregation induced by nonsense mutations due to expression of an "unexpected" N-term truncated protein.

P0700. Molecular diagnosis of fragile X syndrome in mentally retarded patients from Latvia

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Fragile X syndrome (FXS) is the second most common cause of mental retardation (MR). FXS is mainly caused by massive expansion of CGG triplet repeats located in the 5'-untranslated region of the *fragile X mental retardation-1* (*FMR1*) gene. The expanded CGG triplet repeats are hypermethylated and the expression of the *FMR1* gene is repressed in patients with fragile X syndrome, which leads to the absence of FMR1 protein (FMRP) and subsequent MR. Estimate prevalence of FXS is 1 in 4000…6000 males. Normal alleles vary from 6 to approximately 50 CGG repeats. Intermediate alleles 45 - 55 repeats, premutation alleles 59 - 200 repeats (unmethylated), full mutation greater than approximately 200 repeats (methylated).
We analyzed a group of 171 unrelated males with MR referred from clinical geneticists. Molecular diagnostic includes screening by PCR for a normal CGG repeat length and CGG repeat number detection by Applied Biosystems protocol on ABI Prism 310. Three patients with full mutation were detected. The final diagnosis confirmed by Southern blotting with kindly help of prof. K. Eiklid, Oslo, Norway. One allele in intermediate range was detected (50 repeats).
The estimation of AGG inserts structure and ATL1 SNP analysis for further investigation of Latvian FXS patients and their families are in progress.
FXS was detected in 1.75% cases from analyzed patients with MR. We suppose, that number of FXS patients must be higher.

P0701. Molecular studies of FRAXA and FRAXE in S.Indian non-specific MR cases.

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Fragile X syndrome is an X-linked disorder associated with moderate to severe mental retardation. Fragile X A syndrome (FRAXA) and fragile X E syndromes (FRAXE) are caused by trinucleotide expansions in FMR1 and FMR2 genes respectively. Based on the size and methylation status of the expansion, individuals are classified into normal(2-60 CGG repeats), premutated (60-200 CGG repeats) and full mutated individuals(>200 CGG repeats). When the repeat number increases over 200, the gene gets hypermethylated and transcriptionally silenced, hence not producing the FMR protein. We have screened 203 individuals from south India with MR of unknown etiology. All the individuals are clinically evaluated by a 15 item check list. Molecular diagnosis was carried out using PCR technique and sizing of the amplicons were carried out by electrophoresis in denaturing polyacrylamide gel. The cases in which the amplification failed were suspected for the syndrome and were confirmed by southern blot analysis using Stb12.3 probe. Of the 203 cases screened, 7 are full mutated males and 5 carrier females for FRAXA and no cases of FRAXE has been identified. 5 individuals who harbored methylation full mutations for FRAXA were studied for mRNA expressions using RT-PCR and found 2 individuals expressing 2-5 fold increase, 1 individual expressing almost equal and 2 individuals showing less expression in comparison with the FMR1 mRNA levels of normal individuals. This shows that full mutations always do not necessarily focus the transcriptional silencing of the FMR1 gene.

P0702. Basque heterogeneity in the fragile X mutation

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The expansion of a trinucleotide repeat (CGG)n in the FMR1 X-linked gene is the main cause of Fragile X syndrome. Normal alleles consist of tracts of 9-10 CGG repeats interrupted with single AGG triplets. These tracts can become into longer uninterrupted repeats through a gradual slippage or loss of an AGG, leading to unstable alleles prone to expansion to Fragile X mutation. We had previously reported the existence of both types of mutational pathways among general Basque population (Arrieta et al 1999, Peñagarikano et al 2004), despite the absence of the Fragile X full mutation. Basques represent one of the oldest human isolates. The orography of their living area subdivides them into different isolated groups with very little migration until recently. This may have had an effect on allelic frequency distibutions and, therefore, on the prevalence of the associated disorders. With the aim to ascertain any genetic heterogeneity at the FMR1 locus among

Basques. In a previous work (Arrieta et al., 2003) we have analyzed the factors inplicated in CGG repeat stability in a sample from two different and isolated valleys from the Biscay province. The results showed differences between the two valleys in that factors. In the present work we extend the study to a samples from five different and isolated valleys from the Biscay, Guipuzcoa and Navarre provinces within the Spanish Basque region. The data show that differences in allele frequencies as well as in the distibution of the different mutational pathways are present among Basques.

P0703. GAA repeat expansion mutation mouse models of Friedreich ataxia exhibit a slowly progressive neuronal and cardiac pathological phenotype

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Friedreich ataxia (FRDA) is a neurodegenerative disorder caused by an unstable GAA repeat expansion mutation within intron 1 of the *FRDA* gene. However, the origins of the GAA repeat expansion, its unstable dynamics within different cells and tissues and its effects on frataxin expression are not yet completely understood. We have previously reported the establishment of two lines of human *FRDA* YAC transgenic mice that contain unstable GAA repeat expansions within the appropriate genomic context. We now describe the generation of FRDA mouse models by cross breeding both lines of human *FRDA* YAC transgenic mice with heterozygous *Frda* knockout mice. The resultant FRDA mice that express only human-derived frataxin show reduced levels of frataxin, decreased aconitase activity, oxidative stress and age-related neurodegenerative and cardiac pathological phenotypes. These mice represent the first GAA repeat expansion-based FRDA mouse models that exhibit progressive FRDA-like pathology, and thus will be of use in testing potential therapeutic strategies, particularly GAA repeat-based strategies.

P0704. Non-coding repeats expansions and pre mRNA processing defects

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Transcribed non-coding sequences contribute to a correct mRNA formation by providing cis-acting elements that regulate pre-mRNA processing. These sequences are a significant source of human variability and have a mutation potential whose role in causing diseases is largely unexplored. We focused on non-coding GAA repeat expansions in the first intron of the frataxin gene associated with Friedreich ataxia, to unravel basic and pathological mechanisms of pre-mRNA processing. Using hybrid minigenes assay we showed that the repeat expansion affect splice site selection in a position-dependent manner. Repeats insertion downstream a reported exon resulted in its complete exclusion from the mature mRNA. Further insertion at the 3 end or insertions in the upstream intron had no effect on splice site selection. Insertion of control TTC sequences did not affect splicing. In addition, the repeats placed in the first intron of a hybrid minigene, at a location comparable to that found in frataxin gene, unexpectedly resulted in the absence of mature mRNAs in a position dependent manner. 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. Thus the current wisdom that ascribes the defect to a transcription block may have to be revisited. The elucidation of the basic molecular mechanisms that affect pre-mRNA processing in relationship with non-coding repeat expansions will provide a useful insight into a new area with potential for therapeutic intervention in the treatment of this severe degenerative disease

P0705. Epigenetic influences in human diseases; Altered DNA methylation at 4q35 in FSHD1 patients

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Epigenetic mechanisms, which involve DNA and histone modifications, promote changes in gene expression in a heritable manner without directly altering the genome. Methylation of C^5position of cytosine residues in DNA has a great importance in epigenetic silencing. A growing number of human diseases have been found to be associated with aberrant DNA methylation. Facioscapulohumeral muscular dystrophy (FSHD, OMIM 158900) is an autosomal dominant neuromuscular disorder. A contraction of the polymorphic D4Z4 repeat array located at 4q35 locus is associated with the disease. Several observations suggest an epigenetic aetiology in FSHD that causes the transcriptional deregulation of genes close to D4Z4. Van Overveld at al. (2003) has tested this hypothesis by checking the methylation status of the proximal D4Z4 unit at 4q35 and hypomethylation in DNA samples of FSHD patients was detected. Aim of our study was to analyze the methylation state of D4Z4 repeats in 20 Hungarian FSHD patients. Samples from 20 healthy and 10 non-FSHD muscular dystrophy patients were also examined. Genomic DNA was isolated from peripheral blood lymphocytes and digested with methylation sensitive restriction endonucleases (BsaAI and FseI). Southern-blot analysis was performed using P^{32}-labelled p13E-11 probe, specific for 4q35. The intensity of the signals was measured and data were analysed by a Mann-Whitney-U test. Significant difference was detected between the methylation values of samples originating from FSHD patients and healthy individuals, as well as FSHD patients and other muscular dystrophy patients. Our results support the observation that D4Z4 proximal units are hypomethylated in samples of FSHD patients.

P0706. FXTAS in Spanish subjects presenting with ataxias

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Fragile X-associated tremor/ataxia syndrome (FXTAS) is a newly described disorder that occurs in premutation carriers (55-200CGG repeats) in *FMR1* gene. It is characterized by progressive intention tremor, ataxia, and hyperintensities of the middle cerebellar peduncles on T2-weighted MRIs. FXTAS was described in fragile X families, with a suggested age-related penetrance higher that 75% in male carriers of 80 years or older. The incidence of *FMR1* premutated carriers in general population is relatively high and therefore FXTAS might explain a considerably number of sporadic cases of late-onset ataxias. Several studies have been performed in order to determine the real role of FXTAS in undiagnosed patients with movement disorders. The results obtained in European populations ranges from 0% to 4%. We have performed a screening among 144 and 150 unrelated individuals (over 45 years) referred to our laboratory for genetic testing for spinocerebellar ataxia (SCA) or Huntington disease (HD) respectively. In these patients SCA 1, 2, 3, 6, 7, 8, and DRLPA or HD were ruled out. We have identified two patients carrying the permutation (113 CGG and 60 CGG) among the SCA, and none in the HD patients. After MRIs and neurological examination we conclude that the one is a definitive FXTAS while the other is a probable. To our knowledge this is the first study performed with Spanish population and the results obtained demonstrate that Neurologists might take FXTAS into account. (SAF2004-03083, V2003-REDC-07, REDG-098)

P0707. Molecular Identification of the Most Prevalent Mutation of Glucose-6-Phosphate Dehydrogenase Gene in Deficient Patient in Sistan and Balochestan Province of Iran

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Glucose-6-phosphate dehydrogenase (G6PD) in humans is an X-chromosome-linked disorder and housekeeping enzyme, vital for the survival of every cell. G6PD is known to be highly polymorphic from the biochemical characterization of enzyme variants, and more than 380 variants have been found. It catalyses the oxidation of glucose-6-phosphate to 6-phospho gluconate in the first committed step of the pentose phosphate pathway, which provides cells with pentoses and reducing power in the form of NADPH. NADPH is required to protect the cells (via glutathione and catalase) against oxidative damage. G6PD deficiency is one of the most common inherited disorders of mankind

more than 400 million people being affected world wide. In this study we have analyzed the G6PD gene in 92 patients with history of favism. The extracted DNA was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) for known G6PD mutations such as; Mediterranean, Chatham and Cosenza. The results determined that, from the total 92 samples, 74 had G6PD Mediterranean (80.42%) and 2 had G6PD Chatham (2.17%), and Cosenza mutation was not observed (17.43%). G6PD Mediterranean was the most prevalent mutation in Iran and other countries in tropical and subtropical areas. The frequency of Chatham was low in the Sistan and Balochestan province in comparison with other provinces of Iran. In this paper we also try to document the commonly known mutations in patients with G6PD deficiency, with a history of favism.

P0708. Expression of the gamma-glutamyl carboxylase containing the Arg485Pro mutation found in two unrelated VKCFD1 patients

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The vitamin K-dependent gamma-glutamyl-carboxylase (GGCX) catalyses the posttranslational modification of vitamin K-dependent proteins involved in blood coagulation (FII, FVII, FIX, FX, protein C, S and Z), bone and tissue metabolism (BMP and MGP) and cell growth (Gas6). A defect in the GGCX gene results in a very rare bleeding disorder, called “familial multiple coagulation factor deficiency type 1” (VKCFD1). So far, only two different mutations in the GGCX gene (Leu394Arg and Trp501Ser) could be proven to be causative for VKCFD1 by recombinant expression and subsequent measurement of the GGCX activity.

Here we report on the expression of a GGCX variant comprising the Arg485Pro mutation which has been detected in two unrelated patients. GGCX activity assay was performed by adding vitamin K and either the artificial substrate FLEEL in combination with the propeptide ProFIX19, or FIXproGla as a more physiological substrate. Kinetic data show that the Arg485Pro mutation has no effect on FLEEL carboxylation and on vitamin K binding but it strongly affects propeptide binding.

Furthermore, the mutation is located in a highly conserved region of GGCX between amino acid residues 438 and 507 which could previously be demonstrated to comprise the propeptide binding domain.

Our results confirm previous reports on the location of the propeptide binding site of the GGCX and verify the Arg485Pro mutation as causative for VKCFD1.

P0709. Molecular analysis in Gitelman Syndrome: identification of 44 novel mutations in a cohort of 139 patients.

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Gitelman Syndrome (MIM 263800) is an autosomal recessive renal transport disorder, characterized by hypokalemic alkalosis in combination with significant hypomagnesemia and low urinary calcium. Pediatric cases present with transient periods of muscle weakness, fatigue, and symptoms of neuromuscular irritability. The causative gene, *SLC12A3*, encodes the thiazide-sensitive NaCl co-transporter (NCC) in the distal convoluted tubule of the nephron. More than 100 different loss of function mutations in the *SLC12A3* gene have been reported.

In the past few years DNA samples of 139 Gitelman syndrome patients were analyzed by direct sequencing of all 26 exons of the *SLC12A3* gene. In total 76 different mutations were identified, 44 of which have not been reported in literature before. These novel mutations contained mostly missense mutations (30), but also splice site mutations (9), nonsense mutations (3), and deletions (2) were detected.

In 71 patients two mutations were found. In another 20 patients one mutation was detected. In summary, in 65% of patients the diagnosis Gitelman syndrome was supported, and 162/278 (58%) of the alleles have been identified.

Our results indicate that scanning the entire coding sequence of the *SLC12A3* gene leads to the identification of the majority of mutations in Gitelman Syndrome. Since heterozygous exonic deletions are not detected with the currently used protocol, we plan to analyze the incidence of exon deletions in our patient population by multiplex ligation-dependent probe amplification (MLPA). The detection of a homozygous deletion of two *SLC12A3* exons in one patient demonstrates that exonic deletions significantly contribute to Gitelman syndrome.

P0710. Prevalence of the 35delG mutation in the GJB2 gene in Latvian patients with nonsyndromic sensorineural hearing loss

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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.

We obtained DNA samples from patients with prelingual sensorineural hearing loss in whom syndromic forms and environmental causes of deafness had been excluded and their relatives. 37 unrelated patients with sensorineural deafness were screened for the 35delG mutation. This mutation was detected in 45of 74 (61%) tested alleles, 21 patients (57%) are homozygous and three patients (8%) are heterozygous for this mutation. Patients that have 35delG mutation in heterozygous state are suspected either to carry another GJB2 mutation in trans- condition or they have GJB2-unrelated form of deafness. For 13 patients (26%) there was no 35delG mutation found. Four affected relatives of the screened probands found to be 35delG homozygous, one case was detected prenatally. 26 from 50 unaffected relatives of the patients detected to be 35delG carriers. Our findings support the conclusion that the 35delG mutation is the most prevalent GJB2 mutation and that it is the common cause of hereditary nonsyndromic hearing loss in populations of European descent. We are going to continue 35delG molecular testing as a routine method of diagnostics and our next aim is to analyse heterozygous and 35delG undetected patients by direct sequencing to reveal other GJB2 mutations in Latvian patients.

P0711. Molecular screening of connexin 26 : six years of screening

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Several studies have reported that mutations in GJB2 gene (connexion 26, CX26) are a common cause of non-syndromic hearing impairment. More than 100 different mutations of the GJB2 are reported. Many of them are “private” mutations, they have been observed in only one or a few pedigrees, but examples of very common alleles have also been reported in several populations.

We present our findings from the molecular screening of the GJB2 gene over a six years period. Molecular studies were performed sequencing the exon 1 and 2 of GJB2 gene. Over the last 6 years we have studied 2600 individuals primarily Caucasians presenting sporadic or inherited deafness.

Although a high heterogeneity of sequence variation was observed in patients, the 30delG mutation remains the most common pathogenic mutation in our population: 48.6% of genotyped patients presented this mutation in homozygous state and 25.4% in heterozygous state. In the remaining 26% we observed 55 sequences variations included a new nucleotide variation (407insA).

A 342-kb deletion in a gene adjacent to CX26, the GJB6 gene (connexion 30, CX30) has been reported to cause deafness in the homozygous state or in combination with heterozygous mutation in CX26. We decided to perform the analysis of CX30 gene for patients presenting the 30delG mutation in the heterozygous state and a severe or profound hearing loss.

P0712. Molecular study of Glycogen storage disease Type Ia in Tunisia: molecular diagnosis application

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Glycogen storage disease type Ia (GSD Ia, MIM 232200) is an inherited metabolic disorder caused by the deficient of D-glucose-6-phosphatase (G6Pase) activity. GSD Ia is characterized by hypoglycemia, lactic acidemia, hepatomegaly, hyperuricemia, hyperlipidemia and renal dysfunction leading to chronic renal failure as a late manifestation of the disease. The confirmation of the clinical diagnosis is usually performed by liver biopsy. Furthermore in Tunisia the biochemical diagnosis is inaccessible so the molecular investigation may provide a time and cost effective and less invasive tool for diagnosis of GSD Ia. .

Recently, by the study of the molecular basis of GSD Ia in Tunisian patients we have shown that R83C is relatively frequent (10/14 case, 71,42 %). Since the majority of the GSD Ia patients carriers this mutation, a DNA-based diagnostic method was designed for the rapid screening of patients suspected of GSD Ia. This molecular method allows the confirmation of the clinical diagnosis and circumventing liver biopsy and enzymatic investigation. We report here the result of this molecular study and we propose the use of direct screening of this mutation for rapid diagnosis of GSD Ia. by enzyme restriction digestion. The simplicity of the test ensures a short turnaround time.

Since the mutation spectrum in Tunisian patients has now been revealed, the post and prenatal diagnosis of GSD Ia can be made at present by DNA analysis and avoiding liver biopsy.

We propose the use of direct screening of R83C mutation by enzyme restriction digestion as a rapid and valuable tool for diagnosis of GSD Ia in south Mediterranean populations.

P0713. Biochemical and molecular characterization of mutant GM2 Activator deficiency gene in a Turkish family

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GM2-gangliosidosis, AB variant is a recessively inherited lysosomal lipid storage disorder with progressive neurodegeneration leading to death in early childhood. The entity was first defined by Sandhoff in 1971 and localized to chromosome five by Burg et al. in 1985. GM2 activator (GM2A) deficiency can be confirmed by the total absence on the Western blot analysis or by the degradation rate of ganglioside GM2. Since 1991, total of five different mutations in six affected individuals have been reported. The entity seems very rare or underdiagnosed due to limited number of centers performing testing.

We here report the first case of GM2A deficiency from Turkey. 19 mos old girl was evaluated due to the history of neuromotor regression after 5 mos of age presenting with progressive hypotonia, myoclonic seizures, startle response, bilateral macular cherry red spots. GM2 gangliosidosis were the leading entities in the differential diagnosis list; Tay-Sachs and Sandhoff disease were ruled out by normal Hex A/B enzyme levels leaving GM2 activator deficiency as the leading cause. Fibroblasts from the index was tested along with control samples for the degradation rate of ganglioside GM2 and the rate was comparable with controls of Tay-Sachs and AB variant. GM2A protein deficiency was further confirmed by immunoprecipitation analysis. Four coding exons of GM2A gene were all sequenced and a novel 2 base pair deletion in homozygous form was identified in exon two [c.226_227 del CT (p.75fsX37)].

Additional family members were tested for carrier status and prenatal diagnosis was offered.

P0714. Three new mutations in the factor VIII gene

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Hemophilia A, an X-linked bleeding disorder, is caused by mutations in the factor VIII (fVIII) gene. More than 900 mutations within the coding and untranslated region of fVIII gene have been identified. The only common gene defect is intron 22 inversion occurring in 45-50% of severe hemophilia A patients. Moderate and mild phenotypes usually result from missense mutations dispersed through the whole coding region, and are peculiar to individual families. Detection of these point mutations is important for precise genetic diagnosis as well as for studying the genotype-phenotype correlation in this disease.

During molecular characterization of hemophilia A in patients from Republic of Macedonia and Republic of Bulgaria, we have identified three novel missense mutations: Ser558Pro; Tyr2256Asp and Asn90Thr.

Ser558Pro mutation was found in a Macedonian boy with moderate form (fVIII:C 5%) of disease. The replacement of serine by proline, destroys the fIXa binding site which span between codons 558 and 565.

Tur2256Asp missense mutation was found in a Macedonian patient with mild form (fVIII:C 30%) of hemophilia A. This substitution alters the conformation of C2 domain and disturbs the normal factor VIII binding site to phospholipide membranes of thrombocites.

Asn90Thr mutation was found in a Bulgarian hemophilia A patient and his cousin, with moderate form of disease (fVIII 3%). The mothers of both patients are carriers for the mutation, while their maternal grandmother has normal DNA pattern. The maternal grandfather was not available for analysis, however clinically normal, thus suggesting a “de novo” fVIII mutation in grandfather’s germ cells.

P0715. A novel F8C acceptor splice site -3 C>G mutation leads to a 2 base-pair mRNA frameshift and severe hemophilia A

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Hemophilia A is an X-linked bleeding disorder, caused by deficiency in the activity of coagulation factor VIII due to mutations in the F8C gene. The most common defects in patients are inversions involving F8C intron 22 which account for approximately 45% of individuals with severe Hemophilia A. A number of splice site mutations in severe or moderate Hemophilia A have been reported in the literature, however, in the great majority of cases, the effect of the mutation on mRNA splicing has not been experimentally validated. In this study, we identified a novel acceptor splice-site mutation in intron 8 (c.1272-3C>G) in two brothers with severe Hemophilia A. Expression and analysis of a mini-gene construct with the -3 C>G substitution in a transfected COS cell model system demonstrated use of a novel acceptor splice-site two bases upstream, leading not to exon skipping but to a 2 bp frameshift in the FVIII mRNA and proving the causative nature of the mutation.

P0716. Novel *SLC6A19* coding mutations: Hartnup disorder is allelically heterogeneous

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Hartnup disease with symptoms ranging from asymptomatic aminoaciduria to severe neurological and dermatologic manifestations, has been proposed to be genetically heterogeneous, perhaps influenced by either modifier genes or environmental factors, including diet[1, 2]. So far, 10 mutations in a single novel gene, *SLC6A19*, have been shown to segregate in a recessive manner in unrelated families with Hartnup disorder originating from Caucasian and Japanese backgrounds[3, 4]. However, two causative mutation alleles were not detected in *SLC6A19* in all affected individuals and no other genes are reported to be involved. The initial incorrect sequence of *SLC6A19* in the public database prompted us to re-sequence all the coding regions in the families with only a single disease allele identified in this gene[3]. We also investigated four newly acquired families - 3 Australian and 1

Canadian. We found 4 novel missense mutations, all of which are at highly conserved sites in fugu, mouse and human and observed the common D173N allele in 3 of the novel Hartnup families. All affected individuals present with compound heterozygosity for mutations in *SLC6A19*. We also screened 500 healthy controls to establish the frequencies of these new alleles in the healthy population using either PCR-RFLP or TaqMan® assay-by-design. Thus, we explain Hartnup disorder in all our investigated families with allelic heterogeneity at a single locus, confirming the recessive model of inheritance.

1. ScriverC et al, 1987 Am J Hum Genet40:401
2. BröerS et al, 2005 Biochem Soc T33:233
3. SeowH et al, 2004 Nature Genet36(9):1003
4. KletaR et al, 2004 Nature Genet36(9):999

P0717. Genetic Hemochromatosis (GH) in African mixed populations

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Due to history and slave trade, the population of the Reunion Island is originated in part from Europe and from Africa and highly mixed. Ferroportin (FPN) is a membrane protein involved in iron homeostasis and encoded by the SLC40A gene (2q32). It is involved in the transfer of iron from enterocytes or macrophages to the circulation upon the control of Hepcidin. Mutation of FPN gene are associated with ”hemochromatosis type 4” (HFE 4 :OMIM : 606069) which is characterized by a dominant transmission and variable biological patterns. HFE 4 is suppose to be one major genetic determinant of GH in African population. Denaturating HPLC was used to screen SLC40A gene mutation in a large “white Creole” family and where a severe iron overload was diagnosed in 6 patients from 9 persons and over 3 generations. We found that, two already characterized SLC40A mutations (G490D and Q248H) were respectively carried by the grandmother and the grandfather and transmitted in the family. No HFE 1 genotype were found in the family. Biological phenotypes at diagnosis for heterozygotes were comparable with medium ferritin level of 4200 mg/L and TfSat : 28.4 %.One patient was found to be compound heterozygote for the two mutations and displayed a ferritin level much more higher than in other patients : 12620 mg/L and TfSat : 36.8 %. This observation suggest that : 1- FPN mutation behalf in a semi-dominant manner.2- It is likely that SLC40A mutations are frequently involved in GH in such populations.

P0718. The first observation of the hyperunstable Hb Taybee (α1cd38/39delACC; Thr>0) in Greece and 3 new cases with the rare hyperunstable Hb Heraklion (α1cd36/37delCCC; Pro>0): distinct phenotypic expression when co-inherited with α⁺-thalassemia

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Alpha-thalassemia mutations are of the commonest in humans. Amongst >80 α-thalassemia mutations, more common are deletions, partially or completely removing the α-globin gene cluster (16p13.3, ζ2-ψζ$_1$-ψα$_2$-ψα$_1$-α$_2$-α$_1$-θ). Point-mutations within either α-globin gene (nondeletion mutations) are less common. Nondeletion mutations usually reduce α-globin synthesis by impeding RNA processing or translation, but some cause post-translational hyper-instability of the abnormal polypeptide, mimicking α-thalassemia through an overall reduction of α-globin synthesis. Interaction of nondeletion α-thalassemia determinants usually causes HbH disease, a chronic moderate anemia in which excess β-globin chains form HbH (β4). We observed 6 Greek cases with an atypical thalassemia phenotype: chronic moderate anemia without abnormal hemoglobin fractions. DNA analysis characterized common α⁺-thalassemia mutations in-trans to in-frame 3bp deletions in the α1-globin gene: 4 cases (2 unrelated children and 2 adult siblings) had codon36/37,delCCC (Hb Heraklion), and a further 2 unrelated adults had codon38/39,delACC (Hb Taybee). To date Hb Heraklion has been observed in a single Greek case and Hb Taybee in sporadic Israeli-Arab cases. Three Hb Heraklion cases with the nondeletion IVS-1 donor splice-site mutation (α^{Hph}α) in trans had Hb levels 7-9.5g/dl; the fourth Hb Heraklion case and both Hb Taybee cases had α⁺-thalassemia 3.7kb deletion in trans, maintaining Hb levels around 10.5g/dl. All cases had slight to moderate hemolysis (bilirubin 3-7x normal) and increased bone-marrow-activity indicating dyserythropoiesis (serum erythropoietin and transferrin receptor levels 3-5x normal). Absence of detectable abnormal hemoglobin fractions (includng Hb H) in such cases confounds diagnosis based on hematology alone, and definitive diagnosis is only achieved through DNA analysis.

P0719. Recombinant expression fo C1-Inhibitor protein variants and 3-D modelling

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C1-Inhibitor (C1INH) is a single-chain glycoprotein of 105 kDa that belongs to the family of serine protease inhibitors (Serpins).
Hereditary Angioedema (HAE) (OMIM: 106100), an autosomal dominant disease with incomplete penetrance occurs due to mutations in the *C1INH* gene. C1INH deficiency may result in recurrent episodes of acute, local, circumscribed edema of the skin or mucosa.
Five years ago, we started a routine genetic testing protocol for HAE. Over the years, we have analysed DNA samples from almost 400 patients suspicious for HAE. About 30% of our patients present with amino acid substitutions. Since there are some known polymorphisms in the gene which also lead to the substitution of amino acids (e.g. V458M) it is impossible to predict the causality of the alterations offhand. Therefore, we have tested for the functional effect on C1INH activity of a series of C1INH mutations in a recombinant expression system (HEK-293-cells). Most substitutions studied so far resulted in an almost complete loss of inhibitor activity of the recombinant proteins while some mutations (e.g. A-21V and S233T) retained a reduced activity when compared to wildtype protein.
Since the crystal structure of C1INH has not been elucidated yet, we used data from other serpins to build an analogous 3-D model of the protein. This model was used to predict whether the observed amino acid substitutions introduce significant changes in the protein structure.
In summary, recombinant expression of mutated C1INH protein is a useful tool to characterize the role of individual amino acid residues for C1INH activity.

P0720. Rearrangements detected by QMPSF, MLPA and CGH array represent a common cause for holoprosencephaly, leading to a novel diagnosis strategy

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Holoprosencephaly (HPE) is a common birth defect (1/16.000 live births; 1/250 conceptuses) caused by the failure of anterior ventral midline formation, and affecting the forebrain and the face. Screening of the four main genes (*SHH*, *ZIC2*, *SIX3* and *TGIF*) by DHPLC-sequencing and QMPSF revealed point mutations in 18% and microdeletions in 7% of HPE cases. This relative high frequency of microdeletions in the main HPE genes incited us to search for candidate HPE genes using deletion cartography.
As two of the main HPE genes and six other candidate loci are located in subtelomeres, we first searched for microrearrangements in these regions using SALSA MLPA kits (MRC-Holland).

This analysis in 100 foetus and 140 live-born infants with HPE revealed subtelomeric rearrangements in known HPE loci (7q, 18p, 1q, 20p, 21q) but also in novel loci (1p, 5q, 7p, 8p, 9q, 17q, 18q and 22q). Some of these deleted regions (7p, 7q, 1p) were narrowed by quantitative PCR.
A number of samples consisted of an association between a duplication and a deletion in two chromosomal subtelomeres, like (7pdup;7qdel). The finding of such double rearrangements in a same patient reinforces the multigenic and multihit origin already evocated for HPE and will also offer another explanation for the wide phenotypic spectrum described in this developmental disorder.
So the search for microrearrangements must be integrated into the diagnosis of HPE, and the first results obtained by CGH array prove the relevance of this pangenomic approach to identify loci of novel candidate genes.

P0721. Evidence for a critical role of the modifying subunit of glutamate-cysteine ligase in homocysteine pathophysiology

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Background Hyperhomocysteinemia (HHcy) is associated with impaired endothelium-dependent vasodilatation and an increased risk of atherosclerosis and thrombosis. Despite intensive research, the pathophysiology of this association remains conjectural. To unravel the mechanism involved we performed gene-expression profiling in aorta of diet-induced hyperhomocysteinemic rats.
Methods and Results Rats were fed a high-methionine diet in combination with a low B-vitamin diet to induce hyperhomocysteinemia. Application of oligonucleotide arrays demonstrated that the modifying subunit of glutamate-cysteine ligase (GCLM), which is involved in glutathione synthesis, was 2.7-fold up-regulated in the aorta of HHcy rats compared to control rats. In addition, we showed by HPLC analysis that total levels of the anti-oxidant glutathione were increased in serum of HHcy rats. As GCLM has been shown to be up-regulated in conditions of oxidative stress, we hypothesized that a hampered up-regulation of GCLM increases the risk of vascular diseases. To test this hypothesis we examined a functional single-nucleotide polymorphism (SNP) in the human GCLM gene (-588 C>T) in a case-control study consisting of 185 venous thrombosis patients and 500 controls. We demonstrated that the presence of the -588 T allele increased the risk of venous thrombosis more than 2-fold when also homocysteine levels were elevated (OR 2.5 [1.04-6.11]), which confirmed our hypothesis.
Conclusions These data suggest that adequate functioning of GCLM is required as a compensatory mechanism for protection against the adverse oxidative effects of elevated homocysteine on the vascular wall, and thus describe a previously undefined role for GCLM in homocysteine toxicity.

P0722. Development of a UK genetic diagnostic service for limb development disorders

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Congenital abnormalities of the limb occur in 1 in 500 livebirths as isolated malformations or as part of a syndrome. The molecular bases of many of these disorders have been elucidated and the Oxford Molecular Genetics Laboratory is now developing a genetic diagnostic service for a number of these limb development disorders to aid clinical management and genetic counselling of patients and their families.
Diagnostic services have been set up for mutations of *ROR2*, *HOXD13* and *GLI3*. Mutations within *ROR2* cause 2 disorders; Dominant brachydactyly type B1, characterised by aplasia or hypoplasia of the terminal portions of the digits, and recessive Robinow syndrome, a severe skeletal dysplasia with abnormalities of the ribs and vertebrae, dysmorphic facies and genital abnormalities. Expansions, truncating and missense mutations within *HOXD13* can cause a variety of disorders; Synpolydactyly is an insertional digit duplication associated with syndactyly and brachydactyly types D and E involve shortening of digits. *GLI3* disorders have distinct clinical features but all have polydactyly; Pallister-Hall syndrome is characterised by insertional polydactyly, hypothalamic hamartoma and bifid epiglottis, Grieg Cephalopolysyndactyly has preaxial polydactyly, wide big toes and thumbs, syndactyly and macrocephaly/hypertelorism, and postaxial polydactyly type IV is an isolated polydactyly.
Testing involves sequencing of specific hotspots or whole genes depending upon the disorder. At present dosage testing for *GLI3* is being developed, together with a mutation screening service for *FLNA*, mutations of which lead to a range of severe congenital disorders involving limb abnormalities.

P0723. Mitochondrial Deletions in Huntington disease

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Huntington disease (HD) is as a neurodegenerative disorder with autosomal dominant inheritance is characterized clinically by behavioral disturbance, chorea, dementia and ataxia . HD is caused by expansion of the CAG repeat in huntingtin gene. Actual function of huntingtin is not clear but its role is cofactor and mediators in neuronal cells. Also huntingtin is a highly conserved protein and expression is required for normal development. Nowadays oxidative stress effects have considered in pathogenesis associated with HD.
In this study mixed individuals of HD patient and who just showed clinical manifestation screened for four defined mtDNA deletions (*A*, *B*, *C* and *D*).
We found one of *A*, *C* and D mtDNA deletions in at least 60% of samples , no one showed deletion B. 10% showed both *A* and *D* deletion and 30% showed *A* deletion that all of them had expansion allele. Also *C* mtDNA deletion has been observed in 18% of both samples of HD and non HD individuals.
Mitochondrial dysfunction could explain that recorded symptoms from several different organs may be the first manifestation of mtDNA deletion disorder. As well as complication followed by mitochondrial deletion in HD; Its likely that mitochondrial dysfunction in HD is caused by excitotoxicity and the consequent generation of NO and free radicals, with inhibition of Complexes II/III and aconitase by NO and ONOO. This would account for the severity of the defect reflecting the pathological involvement in HD.

P0724. Symptoms and aggregate distribution in Huntington's disease

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Huntington's disease is an autosomal dominant genetic disorder where an expansion in a stretch of CAG repeats in the IT15 gene is translated into an expanded stretch of glutamines in the mutant huntingtin protein. Insoluble protein aggregates have been considered a pathological hallmark of Huntington's disease and other polyglutamine disorders. In this study the number of aggregates was assessed by immunohistochemistry in the superior frontal gyrus and motor cortex of 7 Huntington's disease cases. The number of aggregates was compared to the symptoms (motor/mood) these patients displayed during the course of the disease. Clinical data were collected retrospectively from family members using a semi-structured interview and a questionnaire. Regardless of the pattern of symptoms present in the patients, there was a consistently higher number of nuclear and non-nuclear aggregates in the superior frontal gyrus than in the motor cortex. The regional difference in density of aggregates was not reflected in the variable symptomatology between cases, which questions the significance of the localization of aggregates as an index of pathogenesis and neurological deficit in Huntington's disease.

P0725. Giant and bi-focal forms of adenomatous hyperplasia in congenital hyperinsulinism

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Congenital hyperinsulinism (CHI) may associate histologically with either diffuse insulin hypersecretion or focal adenomatous hyperplasia. Whereas diffuse CHI is of autosomal recessive inheritance, focal CHI is sporadic. Germ-line, paternally-inherited, mutations of the *SUR1* or *KIR6.2* genes (encoding sub-units of the pancreatic ATP-dependent potassium channel) together with somatic maternal haplo-insufficiency for 11p15.5 were shown to result in focal CHI. Unusual focal forms or pluri-focal CHI are rare, and the underlying determinism has not been thoroughly investigated.

We here report two patients with bi-focal CHI as evidenced by relapsing hypoglycaemia following removal of the first focal lesion and the detection of a second, distinct, focal adenomatous hyperplasia to a later surgery (Patients 1 and 2), and a patient with a giant focal lesion involving the major part of pancreas (Patient 3).

In the three patients, a germ-line, paternally-inherited, mutation of *SUR1* was found. In Patients 1 and 2, haplo-insufficiency for the maternal 11p15.5 region resulted from a somatic deletion specific for each of the focal lesions, as shown a diversity of deletion breakpoints. In Patient 3, an identical somatic maternal deletion was shown in two independent lesion samples, as shown by similar breakpoints, suggesting a very early event during pancreas embryogenesis.

Conclusion: The rare patients with pluri-focal or other variant focal CHI may follow the same paradigm as focal CHI with small solitary lesions. Independent somatic events or a early event during pancreas embryogenesis provide a rationale for the observed histological diversity within the focal forms, and for apparent failure of surgery.

P0726. Structural alterations on chromosome 12p in autosomal-dominant hypertension with brachydactyly: Interphase FISH sheds light on the darkness

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The aim of our study is to identify the molecular basis of autosomal-dominant hypertension, brachydactyly type E, and short stature. The hypertension resembles non-salt-sensitive primary hypertension in the general population. Untreated affected individuals die of stroke at age <50 years. The gene(s) reside on a 3.15 Mb, chromosome 12p region. We sequenced all known and predicted protein-coding genes to no avail. However, interphase fluorescence-in-situ-hybridization (iFISH) studies, using a five-clone BAC array spread across the locus, showed an inversion, deletion, and reinsertion mutation in lymphoblastoid cell lines in the original Turkish family. We now extended iFISH-analysis to three other families and one sporadic case (all non-Turkish), with a 24-clone, contiguous BAC array. We confirmed complex rearrangements at chromosome 12p. Surprisingly, all affected persons had an inversion of the centromeric part of the locus with a shared region. However, there were no common breakpoints and no disruptions of any known coding sequences. The sporadic case also had a partial deletion of the inverted sequence that was reinserted in the telomeric part of the locus. We conclude that chromosome 12p rearrangements are responsible for the hypertension-brachydactyly syndrome. Possible causes remain unknown genes or non-coding RNA. A position effect must also be considered that could alter the interplay between a regulatory element and its respective gene.

P0727. Genetic study of spanish families affected by hypertrophic cardiomiopathy

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Background Hypertrophic Cardiomyopathy is typically an autosomal-dominant disease characterized by left ventricular hypertrophy and myofibrllar disarray. The clinical and pathological manifestations are diverse, and range from asymptomatic clinical courses to severe heart failure and sudden cardiac death. Genetic data suggest that 50-60% of the adults with HCM have mutations in one of eigth genes that encode different components of the cardiac sarcomere: β-myosin heavy chain, cardiac Troponin-T, cardiac Troponin-I, α-tropomyosin, cardiac myosin binding protein C, essential and regulatory myosin light chains and cardiac actin.

Results Here, we present the preliminary results of frequencies and types of mutations in 58 unrelated patients with typical phenotype of HCM. In all patients a systematic mutation screening was performed in three sarcomeric genes for which HCM mutations have been described most frequently (MYH7, TNNT2 and MYBPC3) using the DHPLC and sequence analysis. A population of 50 healthy individuals were also included.

We report three novel mutations and one previously describe mutation present in 9 patients and affecting two genes (MYH7 and TNNT2). Six of our patients harboured the same novel mutation and the other three had different mutation.

Conclusions Most of the sarcomeric protein gene abnormalities are missense mutations that results in a single amino acid substitution within or close to important functional domains. The mutations describe in our study are agree with that, but the frequency distribution of mutations founded in this work suggest the need for a more extensive HCM mutation screening in Spanish population.

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P0728. Mutation analysis of beta myosin heavy chain gene in children and young adults suffering from hypertrophic cardiomyopathy

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Objectives: Hypertrophic cardiomyopathy (HCM) is characterized by asymmetrical septal hypertrophy, rhythm disorders and an increased risk of sudden cardiac death. Genetic origin including mutations of beta myosin heavy chain (*MYH7*) gene with 30% of incidence is common. Our aim was to analyze mutations of *MYH7* gene in children and young adults (< 35 years).

Patients and methods: 19 patients (13 boys, 6 girls, age: 1 month - 28 years, mean: 12 years) suffering from HCM were examined with molecular genetic methods. After isolating DNA from peripheral blood, polymerase chain reaction with primers specific to the head and the hinge region of *MYH7* gene (exon 3-23) was made. Mutation analysis (exon 3, 5-12, 14, 16, 19, 21) was performed using denaturing high performance liquid chromatography, then positive chromatograms were sequenced.

Results: Two mutations were first detected in Hungarian patients. The Val606Met (exon 16) mutation was found in more members of one family. In one member of another family, Arg249Gln (exon 9) mutation was found. In two further patients, 3 silent mutations (exon 3: Thr63Thr, exon 8: Phe244Phe, exon 12: Asp376Asp) and 1 intronic polymorphism (intron 2: base 2477 G/T) were also detected.

Conclusion: We could detect Val606Met and Arg249Gln mutations in Hungarian patients for the first time. Knowing the confirmed genetic etiology in children and young adults and having the possibility to analyze the *MYH7* gene with specific and sensitive methods, it is advised to make molecular genetic examination in children suffering from HCM, especially if the disease shows familial occurrence.

P0729. Hereditary Hypophosphatemic Rickets with Hypercalciuria (HHRH) is caused by mutations in the sodium/phosphate cotransporter gene SLC34A3

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There are several hereditary disorders that result in isolated renal phosphate wasting, including X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets (ADHR), and hereditary hypophosphatemic ricktes with hypercalciuria (HHRH). The genes mutated in XLH and ADHR have been identified as PHEX (The HYP Consortium, Nat Genet 11, 1995) and FGF23 (The ADHR Consortium, Nat Genet 26, 2000), respectively.

HHRH is a rare autosomal recessive form that is characterized by reduced renal phosphate reabsorption, hypophosphatemia and rickets. It is distinct from other forms of hypophosphatemia by increased serum level of 1,25-dihydroxyvitamin D resulting in hypercalciuria. Using SNP array genotyping, we mapped the disease locus in 2 consanguineous families to chromosome 9q34. The candidate region contained a renal sodium/phosphate cotransporter gene, SLC34A3. Sequence analysis revealed disease-associated mutations in 5 families including 2 frameshift and 1 splice site mutation.

We also show that the circulating phosphaturic factor FGF23, which is increased in XLH and carries activating mutations in ADHR, has normal to low normal serum levels in HHRH patients, further arguing for a primary renal defect. Identification of the gene mutated in HHRH adds the protein SLC34A3, to the group of proteins - PHEX, FGF23, SLC34A1, and GALNT3 - involved in the regulation of phosphate homeostasis.

We found no mutation in PHEX, FGF23, and SLC34A3 in 4 families with autosomal recessive inheritance of hypophosphatemia, suggesting further heterogeneity. Carrying out a genome-wide scan in these families should result in the identification of more genes involved in phosphate metabolism.

P0730. Molecular characterization of NEMO abnormalities in spontaneously aborted IP males foetuses

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Incontinentia pigmenti (IP) is an X-linked dominant genodermatosis, lethal for males during embryogenesis. The gene responsible for IP encodes the NF-kB essential modulator (NEMO) protein. An identical genomic deletion within *NEMO* accounts for about 75 % of IP females. Small mutations were described in a small portion of IP females. The frequent deletion as well as most of these mutations result in total abolition of NF-κB signalling. Punctual mutations may be easily missed, because of the heterozygosity of IP females and the presence of NEMO pseudogene. Samples from foetal IP males are rarely available. Here we describe the study of NF-κB signalling and molecular analysis of NEMO gene in fibroblasts derived from two unrelated IP male foetuses that aborted spontaneously. Interestingly, in both foetuses not carrying the frequent deletion of *NEMO* gene, we observed a total abolition of NF-kB activation. In foetus 1, we detected a truncated NEMO protein. RT-PCR showed deletions of exons 4, 5 and 6 and sequencing of *NEMO* gene revealed a splice mutation (IVS4+2 T/G). In foetus 2, we detected no *NEMO* protein. Molecular characterization of NEMO by RT-PCR and DNA sequencing is still underway. We hypothesize that in some cases, IP may result from another kind of rearrangement involving either repeated sequences within the *NEMO* gene or the highly homologous NEMO pseudogene. The identification of such rearrangements is impossible in affected females. Males foetal cells expressing the IP defect are thus very useful for identification of new mutations or deletions in NEMO gene.

P0731. Common polymorphism in MTHFR gene and etiology of Leber hereditary optic neuropathy (LHON).

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Medical Genetic Group, National Institute for Genetic Engineering and Biotechnology, P O Box: 14155-6343, Tehran, Iran. Tel: 0098-21-4580383; fax: 0098-21-4580395, LHON is an inherited form of bilateral optic atrophy and loss of central vision. The primary cause of neuropathology and vision loss is mutation in the mtDNA but unknown secondary genetic and/or epigenetic risk factors are suggested. This is the first study has examined folate gene alterations as possible genetic risk factor for LHON. Frequencies of common mutations of C677T and A1298C of the MTHFR gene have been tested in 14 unrelated LHON patients and 145 normal controls. Strong linkages have observed among LHON syndrome and C677T MTHFR ($P<0.000$, $\chi^2>25$) mutation. However, no significant linkage was found between A1298C MTHFR mutation and LHON syndrome. A relationship between C677T MTHFR mutation and LHON neural degeneration etiology can be speculated. This finding help in better understanding of mechanism involve in neuropathology of vision loss and treatment of LHON patients.

P0732. Analysis of complete mitochondrial genomes from Finnish LHON pedigrees

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Leber hereditary optic neuropathy (LHON, MIM #535000) is a maternally inherited disease that leads to bilateral visual failure in young adult life. The majority (95 %) of LHON patients have one of three mtDNA point mutations (3460G>A in *MTND1*, 11778G>A in *MTND4*, 14484T>C in *MTND6*) in genes coding for complex I subunits of the oxidative phosphorylation system. In addition to these primary mutations several additional mtDNA mutations have been identified. Men are more likely to be affected as approximately 77 % of patients are males. The penetrance of LHON is 27 % for men and 8 % for women.

The aim of this study is to elucidate the role of mtDNA variation in the etiology of LHON. We have sequenced the entire mtDNA from 35 Finnish LHON patients (19 with 11778G>A in *MTND4*, 4 with 3460G>A in *MTND1*, 1 with 14484T>C in *MTND6*, 1 with 9101T>C in *ATPase6*, and 10 with no primary mutation). Eight patients were found to belong to haplogroup J. We aim to clarify whether the amount of mtDNA variation is increased in LHON patients. Sequence analysis may also reveal mutations that could explain LHON in the 10 families where no primary mutation was found. We are also looking for mutations that might contribute to the low penetrance of LHON in the 11 families where only sporadic cases were identified. We also aim to find out which sequence variants might account for the high penetrance of the *MTND4*/11778G>A and *MTND1*/3460G>A mutations in haplogroup J.

P0733. The Leu33Pro GPIIIa genetic variants and quantity of the GPIIb/IIIa receptors on platelet's membrane are the factors influenced the platelet hyperaggregation

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Epidemiological studies have shown that platelet hyperaggregation may help predict the risk for ischemic heart disease. The aim of our study was to investigate the process that underlies the platelet hyperaggregation. We formed a group of 14 volunteers with known genotypes of Leu33Pro GPIIIa: 8 - 33LeuLeu carriers, 5 - 33ProLeu carriers and 1 carrier of 33ProPro. This group was analyzed on spontaneous platelet aggregation, platelet aggregation in response to ADP and quantity of the GPIIb/IIIa receptors per platelet. For detection of the Leu33Pro variants of the GPIIIa gene the polymerase

chain reaction and endonuclease digestion with MspI were used, the spontaneous platelet aggregation and ADP-induced platelet aggregation was measured by photometric method and quantity of the GPIIb/IIIa receptors per platelet was measured by flow cytometry with monoclonal antibodies. Mean GPIIb/IIIa quantity on platelet surface was (61330±2404). The maximum GPIIb/IIIa quantity per platelet was 82698, while the minimum GPIIb/IIIa quantity per platelet was 50529. The GPIIb/IIIa quantity per platelet correlated with spontaneous platelet aggregation (R=0.56, p=0.07) and ADP-induced platelet aggregation (R=0.52, p=0.07). Furthermore, spontaneous platelet aggregation depended on the GPIIIa genotype: (1.74±0.22) and (2.32±0.27) for LeuLeu genotype and LeuPro/ProPro genotypes, respectively (p=0.06). These results don't achieve statistical significance may be because of small analyzing group and further studies are required. In conclusion, the data obtained in this pilot study have shown that the platelet hyperaggregation may depend on two factors - quantity of the GPIIb/IIIa receptors per platelet and quality of the GPIIb/IIIa receptors due to Leu33Pro GPIIIa genetic variants.

P0734. Molecular characterization of Helios gene isoforms.

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The Ikaros gene family is a group of transcriptional factors (Ikaros, Helios, Aiolos, Eos y Pegasus) which play an important role in lymphoid development. Besides acting as a classic transcriptional factor, this proteins also are involved in chromatin remodelling complexes.

This gene family encodes zinc finger DNA-binding proteins. All the members of Ikaros family produce multiple isoforms via alternative mRNA splicing. Some of them are dominant-negative isoforms with a non functional DNA-binding domain. Those isoforms have been found in patients with acute lymphoblastic leukemia and may have a role in the development of leukemias or lymphomas. However, some of this dominant-negative isoforms are also found in healthy people.

Our group have characterized new Helios isoforms in haematopoietic and non haematopoietic cellular lines. Several of this new isoforms have novel alternatives exons located in differents introns (He.1+2a, He.1+3a,He.1+5a), Moreover we have cloned isoforms that present a criptic process site in the exón 3 (He.1v y He.Δ6v) and a new dominat-negative isoforma named He.S, which is generated by a novel splicing mechanism.

We have studied the intracellular localization and the protein-protein interaction of some of these isoforms. Moreover, we have analized the Histones acetilation status. Our results suggest that He.1v plays an important role in the histones acetilation, specifically in the Histone H3 and confirms that this proteins are involved in remodelling complexes. The comparative study of differents isoforms will allow us to know more about the role of this family in normal lymphoid development and its possible implication in tumor development.

P0735. Large deletion of exons 2 to 8 in the CAPN3 gene, causing LGMD2A in Bulgarian families

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Here we report on two unrelated limb-girdle muscular dystrophy type 2A (LGMD2A) Bulgarian patients compound heterozygous for the 550delA mutation and a large deletion of exons 2 to 8 in the calpain 3 (CAPN3) gene. The mutation screening revealed that both patients were homozygous for the deletion c.550delA. The parents' analysis showed that only the mothers were the mutation c.550delA carriers and the paternity was confirmed with probability 99.999 in both cases. This data draw the suspicion that the fathers should transmit deletions larger than one base and we assumed the presence of deletions covering the whole exon 4.

To check this hypothesis we analyzed both patients on RNA level. The transcribed fragments were separated by agarose gel electrophoresis. The obtained abnormally transcribed fragment, smaller than the normal one, was extracted from the agarose gel and sequenced. A large deletion covering exons from 2 to 8 was detected in both patients. The same deletion has been previous reported [Joncourt et al., 2003, ESHG], which shows that it could be a frequent cause of LGMD2A and most probably it is due to independent mutational events. We hypothesize that such deletions might not be a rare event in this gene, but using the routine sequence analysis exon by exon they are missed.

Both our patients carry exactly the same mutations, but the clinical manifestation of the disease differed significantly. One of them (male) stopped walking at the age of 17, while the other (female) was still ambulant at the age of 21.

P0736. A combined approach of real-time quantitative PCR and DNA sequencing reveals a high prevalence of intragenic deletions in the *LIS1* gene and questions genotype-phenotype correlations in patients with isolated lissencephaly

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Lissencephaly is a term used to describe neuronal migration disorders that lead to absent or reduced gyration and a broadened but poorly organized cortex. The most common form of lissencephaly is isolated, also referred to as classical or type 1 lissencephaly, and results from mutations in the *LIS1* (chromosome 17p13) or *DCX* (chromosome Xq22) genes. The most frequently found mutation for type I lissencephaly is the complete deletion of the *LIS1* gene, detectable by fluorescence in situ hybridization (FISH). In contrast, intragenic deletions in the *LIS1* gene detected by Southern blotting, have been less frequently reported (about 10% of cases). Using DNA sequencing of the coding region to perform mutation analysis in the *LIS1* gene we have detected 8 single nucleotide mutations, 6 of which have not previously been described. In addition, using real-time quantitative PCR to detect intragenic deletions in the *LIS1* gene we have observed deletions in four of 20 patients analysed. The individual deletions span exon 2, exons six to seven, exons five to eight, and exons 10, 11 and the 3' region of the gene. The deletions have been confirmed via long-range PCR or Southern blotting. Mutations affecting only the last (seventh) WD domain of the protein have not previously been described. Four of our new mutations involve exon 11 and WD7, thus illustrating the importance of this repeat domain. Phenotype-genotype comparison in our patients indicates that other factors may be important in determining the severity of isolated lissencephaly.

P0737. MMP2,9 functional polymorphism in chronic HCV fibrosis development

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The degree of liver fibrosis and inflammation is important in patients with chronic hepatitis C (CHC) in terms of therapy as well as prognosis. Quantitative and qualitative changes in matrix protease activity have an important role in extracellular matrix remodeling accompanying fibrosis liver injury so, any factor which affect the MMPs expression due to its effect in speeding the fibrosis process. we collected 120 chronic fibrosis cases caused by pure HCV infection with out HBV or HDV super infection.

We did biopsy for all patients. We analyzed four different functional polymorphism −1575G/A, −790T/G,−735C/T for MMP-2 and C-1562T for MMP9 by RFLP technique. preliminary results showed that -735C/T had very significant relation with fibrosis process in patients grouped in less than 5 years duration of fibrosis whom had grade more than 9/18 from IHA classification or staged more than 2(p<.005).the MMP9 was association was not as significant as MMP2. nut results are going to be be totally ready in less than month.

P0738. Pathophysiological mechanisms of Lamin A/C associated Charcot-Marie-Tooth disease (CMT2B1)

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Lamins, a class of intermediate filaments, are major components of the nuclear lamina, a filamentous network underlying the inner face of the nuclear membrane. A-type lamins are encoded by the same gene: *LMNA*, as a result of alternative splicing. Up to date, at least nine pathologies, described as laminopathies, have been associated with mutations in *LMNA.* One of these, Charcot-Marie-Tooth disease, type 2B1, is an autosomal recessive form of axonal hereditary motor and sensory peripheral neuropathy caused by the mutation c.892C>T in exon 5 of the gene. In order to progress towards understanding the pathophysiological mechanisms underlying CMT2B1, we studied two different models for the disease: human patient's cells homozygous for the c892C>T mutation, and a murine Knock-In model.

First, the role of *LMNA* in maintenance of nuclear integrity was assessed by immunofluorescent studies. Interestingly, no nuclear abnormalities were observed.

Secondly, gene expression studies performed on microfluidic plates (Low Density Arrays) evidenced significant decreased expression levels for several genes, including *LMNA*, as well as genes from the *FOS*, *JUN* and *EGR* families. On the other hand, expression levels of *HMGCR* and *PPARG*, two genes regulated by the adipose transcription factor SREBP1 were increased. These results suggest that disrupting interactions between lamins A/C and SREBP1 induces activation of transcription of SREBP1 targets.

Finally, in Knock-In models, behavioral, morphological and functional analyses are still on process. Results will be discussed and compared to those obtained on patient's cells.

P0739. Mutations in *LMNA, ZMPSTE24 (FACE1)* and clinical heterogeneity in Prelamin-associated segmental Progeroid Syndromes

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LMNA or *ZMPSTE24* (*FACE1*) defects cause several progeroid syndromes, including Hutchinson-Gilford Progeria Syndrome, (HGPS), Mandibuloacral Dysplasia or Restrictive Dermopahty (RD). *LMNA* encodes two major isoforms: Lamin A, obtained through post-translational processing of its Prelamin A precursor, and Lamin C. The mechanism underlying most lamin-associated progeroid disorders is the toxic intranuclear accumulation of truncated or wild type farnesylated Prelamin A, either due to intrinsic Prelamin A defects hampering its post-translational processing or to the absence/loss of function of ZMPSTE24, one of its key post-translational processing enzymes.

We report eight novel *ZMPSTE24* mutations: five homozygous nonsense or splicing null mutations in patients affected with typical RD, one homozygous loss of function mutation in a patient affected with a complex progeroid syndrome combining premature aging and a severe skeletal muscle dystrophy, and two compound heterozygous *ZMPSTE24* sequence variations in a child presenting with progeroid features, lipodystrophy and insulin resistance.

Additionally, we observed 2 novel *LMNA* mutations causing abnormally spliced mRNAs and truncated farnesylated prelamin A, respectively in a 35 year-old patient presenting progeria-like features, and a 7 year-old patient presenting with Progeria and osteosarcoma. Altogether, our data indicate that, rather than the size of the produced Prelamin (truncated or full length), the level of expression of farnesylated prelamin A is the main feature being correlated to the severity of Lamin A-related progeroid syndromes. Finally, we present the UMD-*ZMPSTE24/FACE1* database, whose aim is to provide integrated genotype-phenotype data on *ZMPSTE24*-associated disorders, with transversal informations at the gene, protein, and clinical phenotype levels.

P0740. The *LMNA/C* and emerin mutations effect in laminopathies patients from Russia

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Mutations in *LMNA/C* gene result in some different diseases named "laminopathies". Most frequent of them are Emery-Dreifuss muscular dystrophy which caused by mutations in emerin gene too, and dilated cardiomyopathy 1A. We have studied Russian patients with various laminopathies diagnosis for emerin and lamin A/C mutations and reviled 15 different disorders most of them are unknown previously. In *LMNA* gene 5 mutations are de novo mutations and 4 of them are localize in forth exon which may be "hot region" for mutation in this gene. In family cases we have reviled clinical polymorphism between affected relatives. For mutations influences investigation we have got skin biopsy material from 3 patients and 4 control donors and prepared fibroblasts culture. The lamins allocations were investigated in part of these cells by immunofluorescence method, another part was investigated by electron microscopy and from the third part the RNA for expression analysis was extracted. Using immunofluorescence analysis we have not revealed any differences in lamin A, lamin B and Sc-35 allocations in patients and control cell. We have designed three locuses multiplex system for detection of emerin, lamin A/C and *GAPDH* as control expression levels. In series of 5 repeat experiments we reviled the *LMNA* expression decreased in one patient with amino acid substitution in compare with other patients and control donors. The system for precise mRNA level analysis by real-time PCR method is in progress now

P0741. Candidate genes for LQT syndrome and arrhythmias - mutation screening

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LQT syndrome is a cardiovascular disorder characterized by an abnormality in cardiac repolarization leading to a prolonged QT interval on the surface ECG. There is a significant risk of syncope, *torsade de pointes* and sudden death. Genes mutated in patients with LQT syndrome encode ion channels. Ion channels are proteins embedded in cell membranes. Long QT is associated with two cardiac muscle ion channels: voltage-gated potassium channels and voltage-gated sodium channels. To the first group belong I_{Kr} and I_{Ks} channels. I_{Ks} channels consist of KvLQT1 (KCNQ1) as an α subunit and minK (KCNE1) as a β subunit, while I_{Kr} channels have HERG (KCNH2) subunit and a MiRP1 subunit. To the voltage-gated sodium channels belong I_{Na} channels, one of them is SCN1B. The β subunit can modulate multiple α subunit isoforms from brain, skeletal muscle, and heart. This gene is 9.73 kb long and consists of 6 exons.

The objective of this study is to identify the underlying genetic basis of a person with LQT syndrome anamnesis. We have identified 17 mutations in genes KCNQ1 (11p15.5), KCNH2 (7q35-q36) and KCNE1 (21q22). This basic set of three LQT genes have been extended about next candidate gene - SCN1B (19q13.1) gene. Analysis of all 6 exons has been performed, whereas the exon 1 has been split into two fragments and exon 6 into three fragments. For mutation analysis we use the SSCP (single strand conformation polymorphism) screen method and the automated sequencing.

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P0742. Genome-wide expression profiling of lysinuric protein intolerance (LPI) with microarrays

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Lysinuric protein intolerance (LPI, OMIM #222700) is a rare autosomal resessive aminoaciduria caused by an impairment of amino acid transport in the small intestine and kidney tubules. The disease is caused by mutations in cationic amino acid transporter gene *SLC7A7*. Approximately 30 mutations have been detected world-wide; in Finland, however, all the patients (n=38) share the same homozygous mutation, 1181-2A-->T. The clinical manifestations of LPI are complex, including failure to thrive, protein aversion, nausea and hyperammonemia. Some patients also develop anaemia, pulmonary alveolar proteinosis and immunological abnormalities. The symptoms vary greatly, both within families and amongst patients affected by the same mutation.

To unravel the possible molecular mechanisms behind the clinical symptoms unexplained by the primary mutation, the gene expression profiles of LPI patients were studied using microarray technology. Whole blood RNA (PAXgene) was extracted from 13 Finnish patients and 10 healthy age- and sex-matched controls. Subsequently, the RNA was hybridised on Sentrix HumanRef-8 Expression BeadChips (Illumina) containing probes for over 23 000 genes.

The microarray data were analysed for expression changes between patients and controls (fold-change, t-test statistics). As a result, we discovered 491 up-regulated and 445 down-regulated genes (fold-change limit 0.8; P <0.05). An initial analysis of the biological processes altered by the up- or down-regulated genes was made. Based on these results, the LPI patients' symptoms and their variability can, indeed, be partly deduced from the changes in their gene expression, other than that of *SLC7A7*. Detailed analysis of the altered gene functions is currently ongoing.

P0743. Disruption of the Slc7a7 gene compromises the embryonic growth in the knock-out mouse model of lysinuric protein intolerance.

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Lysinuric protein intolerance (LPI, MIM 222700) is an autosomal recessive defect of cationic amino acid (CAA) transport at the basolateral membrane of epithelial cells of intestine and kidney, caused by mutations of SLC7A7 gene. A constitutive KO of Slc7a7 was generated by random insertional mutagenesis in ES cells (Lexicon Genetics Incorporated, The Woodlands, Texas). More than 400 Slc7a7+/- intercrosses led to only two Slc7a7-/- live animals. At E 16.5 and E18.5 stages, the proportions of Slc7a7 genotypes were found as expected for an autosomal recessive transmission and the Slc7a7-/- embryos were already smaller than controls. Most of Slc7a7-/- pups were lost at birth because of cannibalism. None of the null embryos showed gross morphological abnormalities apart from a generalized developmental delay.

Genes known to have a key role in regulating foetus-placental growth, i.e. Igf1, Igf2, and insulin-like growth factor binding proteins (Igfbps), were studied in SLC7A7-/- embryos by Real-Time PCR. Results showed down-regulation of Igfs in null animals at E18.5 compared with wild-types. Also Igfbp1 and Igfbp4 were down-regulated, whereas Igfbp2 and Igfbp6 were up-regulated in the liver of null embryos compared with wild-types. This expression pattern of Igfbps is similar to that found in other forms of foetal growth failure. This is the first observation which links CAA metabolism and intrauterine growth control mediated by the Igf pathway.

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P0744. Diagnosis of lysosomal storage diseases in Ukrainian patients

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We report our experience of the diagnosis of lysosomal storage diseases. 400 families originating from all regions of Ukraine were referred to our centre for further specific diagnosis. Initial work-up was based on clinical and radiological evaluation of the proband. Lysosomal enzyme assays in peripheral leukocytes were performed according to standard techniques. During the period 1996 - 2005 specific lysosomal enzyme deficiencies was indicated in 85 families. Gaucher disease was diagnosed in 25 families, metachromatic leukodystrophy (MLD) - in 13 families, the mucopolysaccharidoses - in 28 families (9 MPS I, 3 MPS II, 9 MPS III, 6 MPS IV, 1 MPS VI), mucolipidosis II - in 7 families, GM1 gangliosidosis - in 7 families, GM2 gangliosidosis - in 3 families (2 Sandhoff, 1 Tay-Sachs), Fabry disease - in 2 families.

Frequent mutations in the arylsulfatase A gene (ARSA) were screened by PCR-RFLP. The overall frequencies of the "I" allele (IVS2+1) were 20% (2/20) and of the P426L change was 20% (2/20). Pseudodeficiency alleles were found in 2 patients. For other MLD chromosomes the analysis of all ARSA exons and intron/exon junctions with sequence analysis of fragments was performed. We have revealed 4 novel mutations -F247S, D381E, A416V and A469G. Genotype-phenotype correlations in Ukrainian MLD patients were similar to those reported in other Caucasian populations, but also indicated specific characteristics.

The creation of the database of families with LSD patients plays important role in the accurate management of these patients and in study structure and occurrence of this pathology in Ukraine.

P0745. Insight on the mutational process underlying Machado-Joseph disease (MJD/SCA3), through a haplotype study of normal, intermediate and expanded alleles

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The high polymorphic level within the pathological range, but (usually) also regarding normal range alleles, is a feature common to the large group of genetic disorders caused by an oligonucleotide repeat expansion. In Machado-Joseph disease (MJD), although the range limits have been broadened with new allele-sizes described in different populations, intermediate alleles with 45 to 61 CAGs are very rare, and their role on the generation of *de novo* full expansions is still unknown.

To gain insight into this question, we compared intragenic haplotypes of four intermediate alleles with those found in normal (n=18) and expanded (n=10) chromosomes. We have identified 10 new polymorphic sites, six of which were already analysed, as were three SNPs previously described.

The three intermediate alleles associated with an abnormal phenotype shared the H1: TCAAGC-INT-CAC haplotype, whereas an allele with 51 repeats, described as non-pathogenic and stable upon transmission, had the distinct haplotype H2: CTAGGT-INT-GCA - the most common among normal chromosomes (33%). Expanded chromosomes displayed both haplotypes.

The fact that only H1 was shared by the intermediate alleles associated with a disease phenotype, urged us to study this lineage, in further detail, in both intermediate and normal alleles, to search for *cis*-acting factors potentially involved in repeat instability. Moreover, it would be interesting to study an extended flanking region further up and downstream, to test the hypothesis of gene conversion as the possible mechanism underlying the expansion process from intermediate alleles, as previously raised.

P0746. Analysis of genetic factors in male patients affected with infertility

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Introduction. Genetic factors are important causes for male infertility. They include chromosome abnormalities, Y chromosome microdeletions and certain mutations in the cystic fibrosis gene CFTR. Testing for these alterations is important to establish the correct diagnosis and then to assess the risk for the pregnacy. Modern techniques of assited reproduction can help achieve pregnancy, but they also increase the risk of having an affected child. In this study we analyzed infertile male patients, who were potential candidates for ICSI (intracytoplasmic sperm injection).

Methods. We collected peripheral venous blood from male patients who were diagnosed with azoospermia or oligozoospermia. Analysis included karyotyping, detection of Y chromosome microdeletions of 18 loci in 4 multiplex PCR reactions, detection of ΔF508 and R117H mutations with allele specific PCR and detection of 5T/7T/9T alleles in intron 8 of the CFTR gene with the dot-blot method.

Results. A total of 125 patients were included in the study. 20 patients (16%) were carriers of a major chromosome abnormality which mostly involved sex chromosomes, 6 patients (4.8%) were carriers of Y chromosome microdeletions, 8 patients (6.4%) were heterozygotes for the ΔF508 mutation, 4 patients (3.2%) were carriers of R117H mutation and in 12 patients (9.6%) we detected the 5T allele.

Conclusions. In our sample of patients the most important genetic factor is chromosome abnormality followed by mutations in the CFTR gene. High frequency of CFTR mutations indicates that an extended mutation analysis of this gene may be needed to fully estimate risk for cystic fibrosis in offspring

P0747. Sreening of the RYR1 gene for sequence variants associated with malignant hyperthermia susceptibility.

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Malignant hyperthermia (MH) is pharmacogenetic disorder associated with mutations in sceletal muscle sarcoplasmic reticulum Ca^{2+} regulatory proteins. It manifests as a hypermetabolic crisis triggered by commonly used inhalational anesthetics and a particular paralyzing drug. Malignant hyperthermia susceptibility (MHS) is a dominantly inherited predisposition to MH that can be diagnosed by using an invasive diagnostics test on excised muscle bundles, the in vitro contracture test (IVCT). This test, based on the differential contractile response of normal and MH muscle to caffeine and halothane, differentiates between MHS and MH normal individuals and defines a third MH equivocal (MHE) group of unclear status.

Mutations in the calcium channel of sarcoplasmic reticulum, ryanodine receptor type 1 (RYR1 at 19q13) are the major cause of MHS. We sought to develop a reliable genetic screening strategy based on efficient and relatively inexpansive mutation-detection procedures.

Molecular genetic test has been based on RT-PCR approach with use of total RNA extracted from muscles of individuals (N=50) diagnosed as MHS by IVCT. The mutational hot spot RYR1 cDNA regions were amplified in 9 overlapping fragmants and direct sequenced. Known mutations or novel amino-acid substitutions were identified in 25% of studied patients. Relatives of individuals who have been found to have a mutation causative for MH were diagnosed by subsequent detection of the same mutation in blood-extracted DNA.The improvement of RYR1 mutation identification in MHS individuals combined with subsequent noninvasive genetic testing in family members suggests our strategy for molecular diagnostics of malignant hypertermia susceptibility in the future.

P0748. Lack of association between MDR1 gene polymorphisms and drug-resistant epilepsy

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Epilepsy is one of the common neurological disorders affecting 1-2% of population. Despite considerable progress in pharmacotherapy of epilepsy, more than 30% of the patients are resistant to antiepileptic drugs. In recent years, overexpression of multidrug transporters in blood-brain barrier, have frequently been investigated. Although some studies support evidence of an association between the polymorphisms in these genes and the drug resistant epilepsy, there is also some data that indicates there is no association between these two. One of the frequently investigated genes that is found to be responsible for drug resistance in epilepsy is multidrug resistance 1 (MDR1) gene. Instead of single nucleotide polymorphism detection, haplotype analysis of multiple SNPs in the genes involved in drug resistant epilepsy is preferantial. Therefore in this study we aimed to screen the MDR1 gene for the most common polymorphisms C3435T and G2677AT in drug resistant epilepsy patients and control group. Besides analysing these two polymorphisms in MDR1 gene, we also evaluated family histories, treatment regimes and the backgrounds of the patients. Thirty-nine drug resistant epilepsy and 92 control samples were involved in the study. The polymorphism detection was performed by PCR-RFLP and Pyrosequencing from which the same results were obtained. However, we found no associations between C3435T and G2677AT polymorphisms and multidrug resistant epilepsy. Haplotype analysis including these 2 polymorphisms showed no significant association. As a conclusion these 2 MDR1 polymorphisms are considered not to be responsible for the responsiveness to either different or combined antiepileptic drug therapy in our study group.

P0749. Spectrum of *MKS1* and *MKS3* genes mutations in Meckel syndrome

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Meckel syndrome (MKS) is a letal autosomal recessive disease characterized by cystic kidneys, occipital encephalocele, polydactyly, and liver ductal changes. Recently, two genes among the three loci mapped have been identified: *MKS1* on 17q in Finnish kindreds and *MKS3* on 8q in families from Pakistan and Oman.

We report the genotyping of the *MKS1* and *MKS3* genes in a large multhiethnic cohort of 54 independent cases of MKS with 22/54 consanguineous families. Haplotyping at the *MKS1* and *MKS3* loci showed 5/22 and 5/22 homozygous cases respectively.

Sequence analysis of the *MKS1* gene identified the same homozygous mutation in two Palestinian families, suggesting a founder effect. 4/32 non-consanguineous fetuses were compound heterozygotes for *MKS1* mutations. Two cases, from families of French origin, carried the "Finnish major" mutation IVS15-7-35del29.

Sequence analysis of the *MKS3* gene revealed homozygous mutations in one family from Palestine (splice-site change) and Pakistan (missense mutation). 3/32 non-consanguineous cases were compound heterozygotes, carrying at least a truncating allele, originating from France (2) and United-States (1). Interestingly, in the latter, a previous fetus had cystic kidneys and bile duct proliferation but no brain malformation.

Finally, 6 consanguineous families remained homozygous at the *MKS2* locus on 11q.

Our first results indicate that the *MKS1* and *MKS3* genes are each responsible for at least 10 % of MKS cases with various mutations in different populations. One fetus had a *situs inversus*, further arguing for

a ciliary function of MKS proteins. Finally 10 consanguineous families excluded the three loci, suggesting further genetic heterogeneity.

P0750. Mutation's spectrum in the part of EX 10 MEFV gene in Armenian patients with familial Mediterranean fever.

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Familial Mediterranean fever (FMF) is an autosomal recessive disease particularly common in several populations of Mediterranean origin, in which the prevalence reaches as high as 1/200 individual. This disease is characterized by recurring attacks of fever and serositis. Mutations in *MEFV* gene have been identified in patients with FMF. Most frequent of them are localizing in exon 10 and consist of more than 90% of all mutations.

We have investigated 120 Armenian patients living in Russia from 118 unrelated families. Mutation analysis of *MEFV* gene exon 10 sequence between codons 663 and 771 was performed using SSCP method.

In our series 69 patients displayed homozygous or compound-heterozygous *MEFV* mutations. The M694V/M694V genotype was found in 29/69 patients, in 23/69 patients we found M694V/V726A, M694V/M680I in 11/69 patients. In this series, other genotypes were also found: both the M680I/M680I and the M694V/R761H genotypes were observed in two patients, the V726A/M680I was identified in one patient. In 16 cases we revealed only one mutation: V726A/- in 5/16; M694V/- in 6/16; M680I/- in two and one K695R/- heterozygotes. We found no mutation in 35 families.

Allelic frequency and composition of mutations in our group do not reliable differ from Armenians living in Armenia.

P0751. X-linked mental retardation, choreoathetosis and abnormal behaviour (MRXS10) is caused by an unique mutation in an ubiquitously expressed gene

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Systematic mutation screening on the short arm of the X chromosome between markers *DXS8080* and *DXS1190* revealed a silent C-A transversion in exon 5 of the *HADH2* gene in a patient suffering from mental retardation, choreoathetosis, and abnormal behaviour, a syndromic form of XLMR previously described as MRXS10. The disease has been mapped to Xp11 by linkage analysis in a four-generation family with 5 affected males, previously. The detected C/A substitution segregated with the disease and was absent in 1500 tested control X-chromosomes. It causes an aberrant splicing of exon 5 resulting in a reduced level of wild type transcript and an elevated level of an exon 5 lacking variant in the patient. Real time experiments showed that the amount of wild type transcript in the patient was reduced to one third as compared to healthy controls, whereas the amount of the exon 5 lacking variant was 11 fold up regulated in the patient. Additionally the amount of a third transcript variant harbouring a truncated form of exon 5 was 14 times higher in the patient as compared to controls. Finally, *in vitro*-splicing experiments demonstrated that the silent mutation is associated with the aberrant splicing event. Our result is in concordance with the hypothesis that a prominent proportion of the approximately 260 MRX entities might be caused by unique aberrations. Furthermore, aberrations in ubiquitously expressed MRX genes often seem to cause a phenotype restricted to the brain, which might be more vulnerable to such changes than other tissues.

P0752. New Phenotype for Methylmalonyl-CoA Epimerase Demonstrated using a C. elegans Model of Propionate Metabolism

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We have utilized Caenorhabditis elegans to study human methylmalonic acidemia. Using experimental studies and bioinformatics, a full complement of mammalian homologues for the conversion of propionyl-CoA to succinyl-CoA in the genome of C. elegans, including propionyl-CoA carboxylase subunits A and B (pcca-1, pccb-1), methylmalonic acidemia cobalamin A complementation group (mmaa-1), co(I)balamin adenosyltransferase (mmab-1), MMAHC (cblc-1), methylmalonyl-CoA epimerase (mce-1) and methylmalonyl-CoA mutase (mmcm-1) were identified. To verify predictions that the entire intracellular adenosylcobalamin metabolic pathway existed and was functional, the kinetic properties of the C. elegans mmcm-1were examined. RNA interference against mmcm-1, mmab-1, mmaa-1 in the presence of propionic acid revealed a chemical phenotype of increased methylmalonic acid; deletion mutants of mmcm-1, mmab-1 and mce-1 displayed reduced C14-propionate incorporation into macromolecules. The mmcm-1 deletion mutant produced increased amounts of methylmalonic acid in the culture medium, proving that a functional block in the pathway caused metabolite accumulation. Lentiviral delivery of the C. elegans mmcm-1 into fibroblasts derived from a patient with muto class methylmalonic acidemia and cells from a Mut knock-out mouse could partially restore propionate flux. The C. elegans mce-1 deletion mutant demonstrates for the first time that a lesion at the racemase step of methylmalonyl-CoA metabolism can functionally impair flux through the methylmalonyl-CoA mutase pathway; this suggests that malfunction of MCEE may cause methylmalonic acidemia in humans. The C. elegans system we describe represents the first lower metazoan model organism of mammalian propionate spectrum disorders and should be useful to study methylmalonic and propionic acidemia.

P0753. Molecular characterisation of a balanced chromosome translocation identifies MGST2 as a candidate gene for psoriasis vulgaris

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Psoriasis vulgaris is a chronic inflammatory disease with a strong contribution of genetic factors. A recent meta-analysis of published linkage data has identified a major susceptibility locus (PSORS9) on chromosome 4q31. We report on a male patient with a balanced chromosome translocation t(2;4)(p25;q31.1) and familial psoriasis vulgaris. The breakpoint in chromosome band 4q31 disrupts a non-coding RNA gene (CR742434) that overlaps, in antisense direction, the MGST2 gene. MGST2 encodes a microsomal glutathion S-transferase that plays a central role in the synthesis of leukotriene C4 (LTC4). Altered expression of this gene due to the chromosome rearrangement may influence the synthesis of LTC4 and change the balance of pro- and antiinflammatory mediators, thereby predisposing to disorders like psoriasis. Thus, MGST2 is a promising positional and functional candidate gene for psoriasis vulgaris.

P0754. MicroRNA expression in the mouse inner ear and their possible role in hearing and deafness

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During the last years, microRNAs (miRNAs) have been discovered as having important roles in development and disease of plants, insects, nematodes and vertebrates. The involvement of miRNAs in the development and function of specific tissues and systems have been focused on only recently, following the development of new and sensitive methods to measure miRNA expression and identify their targets. Vertebrate ear development may be dependent on miRNAs, as was suggested by recent studies in zebrafish. Our goal is to identify miRNAs that contribute to the development and function of the mouse inner ear and may be involved in hearing and deafness in mammals, as well as their target mRNAs. We used bioinformatics and other prediction tools to identify potential miRNAs that may control inner ear development or function. Using methods to profile the microRNA of the mouse inner ear, such as the 'Invader' assay and expression arrays, we will decipher the expression of candidate miRNAs in cochlea and compare it to their expression in other tissues. Finally, we will confirm the role of these suspected miRNAs in cochlear cell function and attempt to identify correlations to deafness.

P0755. Novel variant of the mitochondrial DNA A7445G mutation in a Hungarian pedigree.

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Mitochondrial tRNA genes have been found to be associated with nonsyndromic hearing loss. A peculiar form is the A→G transition at 7445 of the mitochondrial DNA (mtDNA) which affects the border of COI/Ser$^{(UCN)}$-tRNA genes and has only been characterized in pedigrees.

We detected this mutation in a 12-year-old Hungarian boy. The familial examinations revealed the maternal inheritance, and five family members were affected. Using PCR/RFLP and direct sequencing of blood DNA we characterized the segregation of the mutation.

The mutation was apparently in homoplasmic form in the proband and his mother. The proband had deafness while his mother had no evidence of hearing loss. The mutation was found in heteroplasmic form in the maternal grandmother and in her niece, who are monozygotic twins and one of them has deafness. The mutation's level was approximately 90-95% of the total mtDNA in the grandmother and only 5-10% in the twins. Unlike other families with this mutation, our patients had no palmoplantar keratoderma.

This is the first Hungarian family with A7445G mutation and this is the first pedigree in which homoplasmic and heteroplasmic forms were detected simultaneously. The normal allele was not segregated to the mother and the proband at all; while on the other branch of the pedigree the heteroplasmy was transmitted in low proportion. Despite the huge difference of the heteroplasmy, there were affected individuals on both branches of the family, which suggests that the degree of the mutated DNA alone does not necessarily determine the development of the clinical symptoms.

P0756. Megalencephalic leukoencephalopathy with subcortical cysts, from mutations to function

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is an autosomal recessive cerebral white matter disorder in children. This disease is histopathologically characterized by myelin splitting and intramyelinic vacuole formation. MLC is caused by mutations in the gene *MLC1* and 50 mutations in this gene have been found. Splice-site, nonsense, and deletion/insertion mutations occur throughout the entire coding region. In about 20% of the patients with a typical clinical and MRI picture, no mutations in the *MLC1* gene are found. The absence of mutations can be the consequence of performing standard analysis of genomic DNA. This study shows that cDNA and qPCR analysis are valuable tools to identify some of the missing mutations.

The MLC1 protein has eight transmembrane domains, but contains no other known functional domains and shows no significant homology with proteins of known function. MLC1 is highly conserved between vertebrates. MLC1 is a plasma-membrane protein, which is expressed in leukocytes and in astroglial end feet at the glial limiting membrane of the blood-brain and at the brain-CSF barriers. Interestingly, the expression of MLC1 in astrocytic end feet overlaps with the expression of the dystrophin-associated glycoprotein complex (DAGC). Mutations in merosin, a component of this complex, lead to a muscular dystrophy accompanied by white matter abnormalities. MRI of the brain of such patients shows remarkable resemblance to the MRI of MLC patients. Our data suggest that MLC1 is part of the DAGC.

P0757. Metachromatic leukodystrophy

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Metachromatic leukodystrophy (MLD) belongs to a larger group of lysosomal storage diseases. MLD is caused by deficiency of the enzyme arylsulfatase A and the resulting inability to degrade sulfated glycolipids, especially the galactosyl-3-sulfate ceramides.

MLD is a rare autosomal recessive metabolic disease characterized by a deficiency of arylsulfatase A (ARSA) activity .The estimated gene frequency for MLD is approximately 0.5%,corresponding to an incidance of 1 in 40,000 births.

ARSA deficiency causes intralysosomal storage of cerebroside sulfate in the cells of the white matter of the central nervous system and of the peripheral nerves, leading to a progressive demyelination and a variety of neurological symptoms.Clinically the disease is heterogeneous and different forms are classified according to the age of symptoms (infantile, juvenile, and adult).

The ARSA gene has been mapped to chromosome 22q13.31 and spans approximately 3.2kbp contains eight exons and encodes a protein of 507 amino acid residues with three N-glycosylation sites .

Three most common MLD mutations are c.459+1G>A, c.536T>G (p.Ile179Ser) c.1277C>T (p.Pro426Leu) as well as the pseudodeficiency allele (c.1620A>G).

We investigated mutations in six exons by PCR-sequencing in our patients.

P0758. Hereditary hemorrhagic telangiectasia is caused by the Q490X mutation of the *ACVRL1* gene in a large Arab family: support of homozygous lethality

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In a large Saudi Arabian family with hereditary hemorrhagic telangiectasia (HHT), we identified *ACVRL1* (*ALK1*) nonsense mutation Q490X in 40 HHT patients and three healthy children, but neither in 11 individuals with epistaxis, 41 other healthy family members, nor in 50 healthy unrelated Saudi Arabian controls. Sequence analysis of the entire coding region of the *ACVRL1* and *ENG* genes in five of the 11 epistaxic individuals did not reveal any other disease-causing mutation. Epistaxis seems to be a relatively common phenocopy of HHT in the family under study. One couple, both affected by HHT and carriers of Q490X, had 12 pregnancies. Three of them ended in spontaneous abortion, four in early neonatal death, and only five yielded living offspring, all of which had HHT and were Q490X heterozygous. This observation corroborates previous claims that homozygosity for HHT causing-mutations is lethal.

P0759. Mohr-Tranebjaerg syndrome: A Tim23 knockout mouse as neurodegenerative animal model

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Tim23 protein is a key component of the mitochondrial import machinery. It is part of the Tim23 complex which is responsible for translocation of matrix located protein precursors across the inner membrane. Tim23 is essential in yeast. Tim23p itself is imported into mitochondria with the aid of the DDP1p/Tim13p complex, located in the intermembrane space. In yeast the homologous Tim8/Tim13 complex is not essential. Lack of this complex leads to a cold-sensitive phenotype and impairment of Tim23p import. Mutations in DDP1 in humans are the cause of Mohr-Tranebjaerg syndrome, the so far only known human disease caused by impaired protein import into mitochondria.

We investigated the direct effect of the lack of Tim23 in mouse and created a Tim23 knockout mouse using the gene-trap strategy. The gene-trap vector inserted between exons 6 and 7 of the gene. A LacZ fusion protein is expressed ubiquitinously in Tim23 +/- mice as shown by lacZ histochemistry. Homozygous Tim23 -/- causes early embryonic lethality. Heterozygous Tim23 +/- mice showed reduced levels of Tim23p as determined by immunoblotting. Phenotypic characterization of these mice revealed sex specific impairment of motor coordination, balance and grip strength. The reduced Tim23p levels correlated with a reduced lifespan of Tim23+/- mice. Some heterozygous Tim23 +/-

mice showed alopecia and kyphosis, which are signs of aging, in early stages of life.
Conclusions: Homozygous knockout of Tim23 is lethal in mouse. Reduced levels of Tim23p result in animals with reduced lifespan and impairment of coordination and grip strength.

P0760. Expression and biochemical characterization of novel mutations and polymorphisms in Spanish and Argentinian mucopolysaccharidosis VI (Maroteaux-Lamy syndrome) patients

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Mucopolysaccharidosis VI (MPS VI) or Maroteaux-Lamy syndrome is an autosomal recessive disorder resulting from the deficiency of the lysosomal hydrolase N-acetylgalactosamine 4-sulfatase (arylsulfatase B, ARSB). Mutation analysis in 14 MPS VI Spanish and Argentinian patients resulted in the identification of 9 novel *ARSB* mutant alleles. In the present study, six missense mutations (c.245T>G [p.L82R], c.284G>A [p.Y138C], c.922G>A [p.G308R], c. 937C>G [p.P313A] c.1340G>T [p.C447F] and c.1421T>C [p.L472P]) were transiently expressed in COS-7 cells and 4-sulfatase activity was determined in cells extracts. All mutations resulted in less than 4% of wild-type enzyme activity and most of them showed no enzymatic activity at all. Mutations were expressed in their haplotypic context concerning two non-synonymous polymorphisms present in the *ARSB* gene, p.V358M and p.S384N. Formerly described as a third mutation in some patients, p.S384N is present in several SNP databases and has a minor allele frequence (MAF) of 0.09, as estimated in a Spanish control population including 46 chromosomes. In our expression studies, *ARSB* cDNAs bearing haplotypes p.358M;p.384S and p.358V;p.384N presented an ARSB activity of 70% and 57% respectively, when compared to the most enzymatically active haplotype combination (p.358V;p.384S). When expressing the p.358M;p.384N haplotype, 4-sulfatase activity was reduced to 16%. These data suggest that these two variants should be considered modifier alleles rather than pathogenic mutations. Western blot analysis and subcellular localization studies of the mutations are currently underway.

P0761. Homocysteine levels and MTHFR C677T and A1298C genotypes in patients with myocardial infarction

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MTHFR plays a key role in metabolism of plasma homocisteine. The C677T mutation in methylentetrahydrofolate reductase (MTHFR) gene is accompanied by reduction of enzyme activity and increased homocysteine level.
AIM : We assessed the association between homocysteine levels and MTHFR 677 and 1298 genotypes in young patients with myocardial infarction (MI) under age 45.
METHODS : Sixty-one MI patients and 43 control subjects were included in our study. The MTHFR 677 and 1298 genotype were analyzed using PCR amplification and digestion with restrictive endonucleases Hinf I and Mbo II. Total homocysteine sera concentration was measured using HPLC with fluorescence detection.
RESULTS : The prevalence of the MTHFR C677T genotypes were not significantly different in patients and controls (C/C 47,5% and 46.5%; C/T 39.3% and 39.5%; T/T 14.7 %and 14.9%), and the prevalence of MTHFR 1298 polymorphism were not significantly different in group with MI and controls too. Subject with the MTHFR 677 TT genotype showed higher levels of tHcy compared with C/C ($p< 0.05$) and C/T genotypes ($p< 0.01$) in MI patients and controls (tHcy were 14.7 µmol/l and 14.9 µmol/l respectively).
CONCLUSION : Our results showed the association of MTHFR677 T/T genotype and increased levels of t Hcy in group of MI patients and controls.

P0762. Mucopolysaccharidosis IVA

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Mucopolysaccharidoses, which are also known as mucopolysaccharide storage (MPS) diseases, are a group of rare genetic disorders caused by the deficiency of one of ten specific lysosomal enzymes.
Morquio syndrome (MPS IV) is a mucopolysaccharide storage disease that occurs in two forms: type A , caused by deficiency of lysosomal N-acetylgalactosamine-6-sulfatase, and type B, caused by a deficiency of lysosomal beta-galactosidase. Type A is more common, but the two types generally have the same symptoms. Deficiency of either enzyme leads to an accumulation of keratan sulfate, and bony abnormalities of the head, chest, hands, knees and spine may occur as a result of this metabolic defect. Intelligence is normal.
Mucopolysaccharidosis IVA (MPS IVA; Morquio A disease) is an autosomal recessive . The cDNA contains an open reading frame of 1566 bp which encodes a 522-residue polypeptide. The gene spans approximately 50 kb, and contains 14 exons.The variety (missense or nonsense), frequency, and location of point mutations causing human genetic disease are highly non-random.
We identified 32 different mutations. Transitional mutations at CpG dinucleotides accounted for 23% of all single base substitutions leading to missense and nonsense mutations in the coding region (10 of 44 alleles). Methylation of individual CpG cytosines was extensive within exons 2-14 while CpG cytosines in exon 1 were completely unmethylated. All transitional mutations at CpG sites were located between exons 2 and 14. Non-methylation at CpG sites correlated with the absence of transitional mutations in exon 1.
For molecular diagnosis Sequence Analysis, is recommended.

P0763. Complex I Deficiency in Persian Multiple Sclerosis Patients

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system characterized by the morphological hallmarks of inflammation, demyelination and axonal loss .Until now, little attention has been paid to the contribution of mitochondrial respiratory chain enzyme activities to MS. In this study, kinetic analysis of mitochondrial respiratory chain complex I enzyme (measured as NADH-ferricyanide reductase) was performed on intact mitochondria isolated from fresh skeletal muscle in MS patients (n=10) and control subjects (n=11). Mitochondrial DNA common deletion and deletions were also tested in MS patients. 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. The presupposition of relationship between MS and mitochondrial disorders is due to predominant maternal transmission of MS in affected parent-child pairs, pathoaetiological role of respiratory chain dysfunction in multisystem disorders and important role of it in neurodegenerative disorders, a number of patients such as LHON or other mtDNA abnormality with developed neurological symptoms indistinguishable from MS and similarity of clinical symptoms in mitochondrial disorders to those of MS. This study suggested that a biochemical defect in complex I activity may be involved in pathogenesis of MS.

P0764. Mitochondrial D-Loop variation in Persian Multiple Sclerosis Patients: K and A haplogroups as a risk factors!!

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Multiple Sclerosis (MS) is a multifocal demyelinating central nervous system disorder in which interplay between genes and the environment are supposed to be involved. Mitochondrial DNA (mtDNA) has the only non-coding regions at the displacement loop (D-loop) region that

contains two hypervariable segments (HVS-I and HVS-II) with high polymorphism. mtDNA has already been fully sequenced and many subsequent publications have showed polymorphic sites, haplogroups and haplotypes. Haplogroups could have important implications to understand association between mutability of the mitochondrial genome and disease. To assess relationship between mtDNA haplogroup and MS, we have sequenced the mtDNA HVS-I in 54 MS patients and 100 control subjects. 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).Thus, these two haplogroups might act synergistically to increase the penetrance of MS

P0765. X chromosome inactivation in females with multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disorder and a chronic inflammatory disease of the central nervous system. Loss of immunological tolerance to self-antigens seems to be a common feature of autoimmune disorders. X linked self-antigens could be influenced by X chromosome inactivation pattern, and contribute to the skewed female:male ratio (2:1) in the frequency of MS. A high frequency of skewed X inactivation was recently reported in females with autoimmune thyroid disease, and skewed X inactivation has also been described in women with the autoimmune connective tissue disease scleroderma. In order to investigate a similar potential role of X inactivation in MS, we compared the X inactivation pattern in 125 female MS patients (aged 22-53, median 39 years) with a control group consisting of 117 blood donors (age 19-55, median 38 years). The MS patients were divided into two subgroups according to disease course; primary progressive (PP, n=17) or relapsing remitting (RR, n=108, including 24 females with a secondary progressive course). We found no difference in the median degree of skewing between PP patients (median 60%), RR patients (median 64%) and controls (median 65%)(p= 0.46 median test). The frequency of skewed X inactivation (>80%) was higher in the PP patients (23.5%) compared to the RR patients (14.8%) and controls (12.0%), but the difference was not statistically significant. We conclude that X chromosome inactivation pattern does not seem to be more skewed in MS patients than in controls and does not explain the skewed female to male frequency ratio of MS.

P0766. Congenital Myasthenic Syndrome (CMS): A review of Molecular Genetic Analysis to date

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Congenital Myasthenic Syndromes (CMS) are a group of clinically and genetically heterogeneous conditions caused by defective transmission at the neuromuscular junction, resulting in fatigable muscle weakness. Identification of the causative mutations is important for clinical and family management.

The Oxford Myasthenia centre, supported by the National Specialist Commissioning Advisory Group (Department of Health) provides a National Clinical and Genetic service for the diagnosis of CMS. Full mutation screening is offered for the genes encoding the α, β, δ and ε subunits of the acetylcholine receptor (*AChR*), rapsyn, choline acetyltransferase (*ChAT*) and the collagen-like tail subunit (*ColQ*). Our strategy involves clinical assessment followed by targeted mutation screening by dHPLC WAVE™ and sequencing. Functional analysis to determine pathogenicity of novel mutations is also available.

To date we have received 334 referrals, 247 of which are affected probands. Pathogenic changes have been found in 19% (47 cases, of which 8 are novel mutations). In addition 56 confirmation tests and 49 carrier tests have been performed. We will present a review of data, including a breakdown of the numbers, types and outcomes of referrals, produced to date.

P0767. The pattern of mutations in Feingold syndrome establish a loss-of-function mechanism for the canonical *MYCN* oncogene.

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Feingold syndrome (FS MIM 164280) is characterized by variable combinations of esophageal and duodenal atresias, microcephaly, learning disability, syndactyly and cardiac defect. We have recently established that heterozygous mutations in the *MYCN* gene on chromosome 2p23-p24 are causative for the disorder in 15 unrelated FS families. All mutations were located in exon 3 and disrupt both the full-length protein as well as a novel shortened MYCN isoform, denoted ΔMYCN.

The *MYCN* oncogene consists of three exons. The canonical MYCN protein is produced by the use of an ATG start codon in exon 2 that is preceded by an Internal Ribosome Entry Site. Alternative transcripts lacking exon 2 encode ΔMYCN, an N-terminally truncated MYCN isoform produced by initiation of translation in exon 1. Since all previous mutations affected both the MYCN and ΔMYCN isoforms, it was unknown whether the pathophysiological mechanism was caused by disruption of either one or both of the protein isoforms. Here we describe the identification of 10 additional heterozygous mutations in FS families. While most of the mutations are located in the common exon 3, frameshift mutations were identified in exon 2 in three unrelated families. In addition, a polymorphism was identified in exon 1, which disrupts the predicted open reading frame of the ΔMYCN isoform. Taken together these results demonstrate that multiple aspects of early embryogenesis, postnatal brain growth and tumorigenesis in humans are tightly regulated by dosage of canonical MYCN protein and that heterozygous disruption of ΔMYCN is not associated with any obvious phenotypic abnormalities.

P0768. Evidence of instability of intermediate number of CTG repeats in *DMPK* in a Norwegian kindred with myotonic dystrophy

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We here report on a kindred descending from eight sibs with no clinically recognized muscular dystrophy. One sister suffered from cataract since the age of 40 and one brother died unexpectedly of cardiac arrest at the age of 67. Mild muscular symptoms came to attention in the next generation and were diagnosed as fibromyalgia, except one case with recognized myotonic dystrophy, where a confirmational Southern blot analysis indicated 130-500 CTG repeats in one *DMPK* allele. The affected patient had a grandchild with a severe juvenile form. One daughter of the cataract patient developed myotonic dystrophy in the presence of a relatively low number (53-61) of CTG repeats. Of the offspring of the patient with cardiac arrest, which could represent a phenocopy, four individuals underwent molecular testing. All of them exhibited an allele with only 40 CTG repeats in *DMPK*. It has been established that myotonic dystrophy is caused by *DMPK* alleles with more than 50 CTG repeats, while the normal number is 5-37 repeats. Intermediate numbers of CTG repeats between 38 and 49 has been considered stable, although rare. The study of this kindred suggests that an allele with 40 CTG repeats only has been able to expand, and that anticipation resulted in a severe phenotype in two generations only and a severe juvenile form after another two generations. We conclude that the so-called intermediate number of CTG repeats must be considered potentially unstable, and that this has consequences for genetic counseling.

P0769. Identification of entire *LMX1B* gene deletions in nail patella syndrome: Final evidence for haploinsufficiency as the main pathogenic mechanism underlying dominant inheritance in man

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Nail patella syndrome (NPS) is an autosomal dominant disorder characterised by nail and skeletal malformations, nephropathy, and glaucoma. Phenotype studies of *Lmx1b-/-* mice revealed nail and patellar anomalies similar to human NPS, which contributed to the identification of mutations in the LIM-homeodomain encoding *LMX1B* gene as the genetic defect. It is as yet unclear why heterozygous *LMX1B* mutations cause NPS in humans, whereas *Lmx1b* heterozygous mice are completely normal. The hypothesis that haploinsufficiency is the main mechanism underlying dominant inheritance in human NPS is based on the fact that the same phenotypic variability is observed in individuals with *LMX1B* missense, nonsense, frameshift or splice-site mutations and that the range and severity of symptoms varies both within and between families. This assumption is supported by the lack of any dominant-negative effect observed by *in vitro* experiments studying missense and truncation *LMX1B* mutations. By MLPA analysis with specific probes for the different exons 1-8 of *LMX1B*, we found a deletion of the entire gene in two unrelated individuals with NPS and a deletion of exons 3-8 in another patient from a series of 3 classic NPS families (3/8; 38%) in which no mutation could be detected by sequencing *LMX1B*. The phenotype of these individuals is comparable with our previously reported families in which *LMX1B* missense mutations were identified. Further characterisation of the size of the deletions is presently being performed. This first identification of entire *LMX1B* deletions strongly confirms the hypothesis that haploinsufficiency is the principal pathogenic mechanism of NPS.

P0770. Phenotype-genotype correlation in patients with Netherton syndrome

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Netherton syndrome (NS) is an autosomal recessive disorder characterized by congenital ichthyosis and severe atopic manifestations. A specific hair shaft defect ('bamboo hair') is diagnostic for the disease. The genetic defect in NS is caused by mutations in the SPINK5 gene that encodes a serine protease inhibitor LEKTI (lympho-epithelial Kazal-type related inhibitor). The phenotype of the patients can vary considerably in severity, and can fluctuate over time in individual patients. The aim of the work presented here is part of an effort to correlate the clinical phenotype of our patients with NS with their genotype.
To this end, the 33 exons, and flanking parts of the introns, were PCR amplified and sequenced. In six of the eight patients studied, two mutations could be identified in each patient, a result that is expected for an autosomal recessive disease. Four of these are homozygotes, probably because of consanguinity of the parents: two carry a nonsense mutation, one patient a frameshift mutation and one a splice site mutation. The other 2 patients are compound heterozygotes for two splice site mutations, or for a splice site and a frameshift mutation. In the two remaining patients only one (a splice site mutation) or no mutation could be found. Phenotypic data are being collected from these patients, and these data will be compared to the type and position of the mutations present.

P0771. Phenotype associated with neurotrypsin mutation: a preliminary report

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The importance of extracellular proteases and their inhibitors is well documented in several human diseases (hemophilia B, alpha-1-antitrypsin deficiency, serpinopathies).
Neurotrypsin belongs to the subfamily of trypsin-like serine proteases. It was localized in the presynaptic membrane and the presynaptic active zone of central nervous system synapses. Neurotrypsin is expressed in the cerebral cortex, the hyppocampus and the amygdala, suggesting a role in learning, memory and emotion.
Neurotrypsin (PRSS12) mutation is associated with autosomal recessive mental retardation. To date, seven patients from three consanguineous families who are all originally from the same area of Eastern Algeria have been identified with the same mutation (4-base pair deletion in exon 7 of the PRSS12), suggesting a founder effect in this population.
Clinical examination revealed impaired voluntary saccadic eye-movements in the horizontal and vertical direction whatever the nature of the stimulation (visual, auditory, somesthesic. Ophthalmological examination including FO and ERG was unremarkable. Ocular movement recording has ruled out ocular motor apraxia.
Intrafamilial clinical expression of neurotrypsin mutation is variable. However, we can distinguish 2 clinical pictures: one is characterized by severe mental retardation, absence of language and autistic features, the other one by moderate global mental retardation without autistic features. Further investigations will be required to objective abnormalities of voluntary eye-movements in these patients.

P0772. Mutated NLGN4 in a family with mental retardation, autism and mood disorder

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Neuroligin 4 *(NLGN4)* belongs to the neuroligin family made up of neuronal cell-surface proteins located in synaptic structures and mostly enriched at excitatory synapses. *NLGN4* mutations were previously associated with mental retardation and susceptibility to x-linked autism. We examined 6 siblings of which 3 males (ages 9 - 14 years) were affected with mental retardation and autistic features and one female (16 years) was diagnosed to have behavioral problems and suspected mood disorder. The familial co-segregation of mental retardation and autistic features in males supported an X linked inheritance. Molecular analysis was performed using markers (DXS1060 and DXS 996) spanning the *NLGN4* site located at Xp22.33. Following suggestive *NLGN4* linkage, we screened the entire coding sequence of this gene and identified a missense mutation N515S. This *NLGN4* mutation was present in the 3 male sibs with mental retardation and autism and also in their sister who presented with behavioral and mood disturbances, yet not in two additional healthy sibs. Screening 100 chromosomes of healthy individuals from a similar ethnic background did not detect this mutation. It may be proposed that not only types of autism and mental retardation but also mood disorders may share a common genetic origin. Familial occurrence of autism and mood disturbances should direct to *NLGN4* as a predisposing genetic factor. These findings may lead to better understanding of molecular pathways and possibly to development of more specific treatment modalities.

P0773. NOD2/CARD15 nonsynonymic single nucleotide polymorphisms in Russian patients with Crohn's disease

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There are two clinical subtypes of inflammatory bowel disease (IBD): Crohn's disease (CD) and ulcerative colitis (UC), which differ from each other by morphological criteria. CD has a strong genetic component, with a lifetime risk of 10-20% for IBD development. Several nonsynonymic single nucleotide polymorphisms of *NOD2/CARD15* gene (16q21) have been shown to be associated with susceptibility to CD.
The DNA samples from 51 unrelated patients with CD and 54 population controls from Russia were investigated for *NOD2/CARD15* gene SNPs: P268S, R702W, G908R and ins3020 C.
The allele frequency for 268S was 0,57 among CD patients and 0,44 among population group (p>0,05) while for 702W it was 0,29 in patients

and 0,02 in population (p<0,0003); for ins3020 C it was 0,49 and 0,08 respectively (p<0,00001) and allele 908R was reviled with frequency 0,08 in affected group and was absent in population. We suggest that P268S substitution is not significant for CD manifestation but all other SNPs may be important for disease development.
Twenty five from fifty one (49%) of CD patients had at least one potential disease-causing polymorphism, whereas only 5 from 54 population persons had it (p<0,000001). Double or more predisposition alleles in homozygous or compound heterozygous status were found in 22% (11/51) affected patients but did not reviled in population.

P0774. Contiguous deletion of the *NDP, MAOA, MAOB* and *EFHC2* genes in a patient with Norrie disease, severe psychomotor retardation and myoclonic epilepsy

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Norrie disease (ND) is an X-linked disorder, inherited as a recessive trait that, therefore, mostly affects males. The gene responsible for ND, called *NDP*, maps to the short arm of chromosome X (Xp11.4-p11.3). We report here an atypical case of ND, consisting of a patient harbouring a large submicroscopic deletion affecting not only the *NDP* gene but also the *MAOA*, *MAOB* and *EFHC2* genes. Microarray comparative genomic hybridisation analysis showed that eleven consecutive bacterial artificial chromosome (BAC) clones , mapping around the *NDP* gene, were deleted. These clones span a region of about one megabase (Mb) on Xp11.3. The deletion was ascertained by fluorescent *in situ* hybridisation analysis with different BAC clones located within the region. Clinical features of the proband include bilateral ocular atrophy, microcephalia, severe psychomotor retardation without verbal language skills acquired, and epilepsy. The identification and molecular characterization of this case reinforces the idea of a new contiguous gene syndrome that would explain the complex phenotype shared by atypical ND patients. (FIS 04/1126, V2003-REDC-07, REDG-098)

P0775. Molecular diagnosis of the neuromuscular diseases in Latvia

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Neuromuscular disorders are diseases that affect the neuromuscular components: the nerve root, the peripheral nerve, the neuromuscular junction and the muscle.
In Latvia DNA testing is available for the 3 most common neuromuscular disorders: X-linked Duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA) and Charcot-Marie-Tooth disease (CMT1A). The molecular diagnosis of Central core disease (CCD) is in progress.
Following methods are used for the DNA testing: multiplex PCR, PCR and restriction enzyme digestion, fluorescent PCR.
DMD is X-linked muscular dystrophy, primarily affecting voluntary muscles, caused by the absence of dystrophin, protein associated with the sarcolemma in skeletal and smooth muscle. Three patients from 13 referred to the molecular diagnostic were confirmed for the DMD diagnosis.
SMA is an autosomal recessive genetic disease caused by deficiency of a motor neuron protein (SMN).
59 patients suspected of having SMA were analysed. Eighteen from them (35,5%) lacked both copies of SMN1, and 6 (10,2%) - both copies of SMN2. Two prenatal samples from the heterozygous patients showed the presence of at least one copy of SMN1. Other patients have no confirmed diagnosis of SMA, but need the quantitative analysis of SMN gene-copy number also as patients with both lacked SMN2 copies.
CMT type 1A is an autosomal dominant neurological disorder that causes damage to the peripheral nerves, carrying signals from the brain and spinal cord to muscles. Eight patients from 13 referred for the molecular diagnostic have confirmed CMT1A diagnosis.
The DNA testing for the CCD is in progress.

P0776. Novel phenotypic variant of the OCTN2 V295X mutation

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In a previous paper we reported two non-consanguineous Hungarian Roma (Gypsy) children who presented with cardiomyopathy and decreased plasma carnitine levels (Melegh et al; Am J Med Genet 2004;131:121.). Homozygous deletion of 17081C of the SLC22A5 gene was detected that resulted in a frameshift at the R282D and lead ultimately to a premature stop codon in the OCTN2 high affinity carnitine transporter (V295X). In both families sudden infant death syndromes were also documented. In a two year old male Roma patient presented with hepatopathy resembling to urea cycle defect we detected the same mutation; there was also sudden death in this family. Taking into consideration that this mutation was already verified in two large families, the recognition of the mutation in a further family suggest a wide ancestral spread of it in certain Roma subpopulations. In addition, identification of a novel phenotypic variant associated with the homozygous deletion of 17081C of the SLC22A5 gene shows that various clinical manifestations can be presented similar to other SCL22A5 mutations.

P0777. Molecular genetic analysis of ornithine transcarbamylase deficient twins.

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Ornithine transcarbamylase (OTC) locus is located in the short arm of X-chromosome. The deficiency of the enzyme is the most common inborn error of the urea cycle caused by a vast number of point mutations, deletions and insertions in the respective gene. The most common form of the disease occurs as hyperammonaemic encephalopathy. The patient is a 32 years old asymptomatic woman, who had four spontaneous abortions before. She became a monozygotic twin pregnant spontaneously. Because of inevitable preterm delivery she gave birth to male twins, who died because of hyperammonaemic state. We took a liver biopsy post mortem from the second boy. Following DNA isolation, it was examined by polymerase chain reaction (PCR) amplification of all 10 exons of the OTC gene including exon-intron boundaries and subsequent single strand conformation polymorphism analysis. The amplimer of exon 8 revealed an aberrant migration pattern and was further analyzed by DNA sequencing. The results indicated a new homologous point mutation of codon 253 (GCA to ACA), which causes replacement of Ala by Thr. We also took her blood sample, isolated the DNA with Roche High Pure PCR Template Preparation kit and analyzed the DNA sample by PCR amplifying and sequencing of the exon 8 of the OTC gene. The DNA sequence data showed that the mother is a carrier of the same mutation that was previously detected in the case of her son. We are planning preimplantation genetic diagnosis in the next pregnancy.

P0778. Consortium for Osteogenesis Imperfecta mutations: Lethal regions in the helical portion of type I collagen chains align with collagen binding sites for integrin and proteoglycans

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To explore genotype-phenotype relationships between mutations in type I collagen genes (COL1A1 and COL1A2, encoding α1(I) and α2(I) chains, respectively) and Osteogenesis Imperfecta, we identified 832 independent mutations, of which 682 (80%) result in substitution

for glycine residues in the helical domain and 150 alter splice sites. Glycine substitutions on either chain that have greater than 5 recurrences are almost always associated with CpG residues. Distinct genotype-phenotype relationships emerge for each chain. One-third of α1(I) glycine substitutions are lethal, especially residues with a charged or branched side chain. Substitutions in the first 200 residues are non-lethal and have variable effect thereafter, unrelated to folding or helix stability domains. Two exclusively lethal regions (691-823 and 910-964) align with Major Ligand Binding Regions, suggesting crucial interactions of collagen monomers or fibrils with integrins, MMPs, fibronectin and COMP. Mutations in COL1A2 are predominantly non-lethal (80%). Lethal substitutions are located in 8 regularly spaced clusters along the chain, supporting a regional model. The lethal regions align with proteoglycan binding sites along the fibril, suggesting a role in fibril-matrix interactions. Unlike α1(I), recurrences at the same site in α2(I) are generally concordant for outcome. Splice site mutations in COL1A1 are rarely lethal; they often lead to frameshifts and the mild OI type I phenotype. In α2(I), lethal exon skipping events are located in the carboxyl half of the chain. These genotype-phenotype relationships indicate that the two collagen chains play very different roles in matrix integrity and that phenotype depends on intracellular and extracellular effects.

P0779. *PANK2* gene mutations in patients with progressive neurodegeneration

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Pantothenate kinase associated neurodegeneration (PKAN; MIM 234200) is a rare autosomal recessive neurodegenerative disorder characterized by progressive extrapyramidal dysfunction, retinopathy, difficulties with speech, and cognitive decline. The accumulation of iron in basal ganglia and their degeneration are detectable by magnetic resonance imaging (MRI) as a sign called ,,eye of tiger". Numerous mutations in the gene encoding pantothenate kinase-2 (*PANK2*) were identified in patients with PKAN. Four homologous pantothenate kinase genes were identified in the human genome, *PANK1-4*, but up to now, only *PANK2* and *PANK4* have been shown to code for proteins with pantothenate kinase activity. In some patients, linkage of the disease to the *PANK2* locus at 20p13 was excluded. To identify the molecular cause of the disease and to confirm the diagnosis of PKAN at the molecular level, we performed DNA sequencing of the coding region of the *PANK2* gene in patients suffering from progressive neurodegeneration accompanied by the typical ,,eye of tiger" sign on MRI. We studied fourteen patients from eleven families and identified mutations on both alleles in eight patients from seven families. In total, we detected eight different mutations. Two mutations were already described: GeneBank AF494409: c.1561G>A (p.Gly521Arg), and c.1583C>T (p.Thr528Met). Six mutations were novel, observed only in the Czech population: c.515_527del13 (pVal172fsX29), c.845_847del3 (p.Leu282del), c.805G>A (p.Glu159Lys), c.1235+5G>A, c.1369G>T (p.Asp457Tyr), and c.1630G>T (p.Gly544Trp). We did not find any *PANK2* gene mutations in the remaining six patients. This can be explained by genetic heterogeneity of the disease.

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P0780. Association study of sporadic Parkinson's disease genetic risk factors in patients from Russia by APEX technology

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Most patients with Parkinson's disease (PD) have sporadic form of the disease with a multifactorial etiology due to interactions between environmental conditions and the genetic constitution of the individuals. We have analyzed by APEX technology 50 single nucleotide polymorphisms (SNPs) in 19 genes related to cholecystokinin, serotonin, dopamine and opioid neurotransmission. Significant differences in the allele and genotype frequencies between the controls and PD patients were detected for 4 SNPs from 3 genes (serotonin 2A receptor, Wolfram syndrome 1, proopiomelanocortin genes). Two SNPs in POMC gene were also associated with different clinical forms of PD. Our data suggest that at least three genes involved in neurotransmitter systems may have more specific role in genetic predisposition to PD.

P0781. Analysis of exon deletions and duplications in PARK2 gene by TaqMan Real-time PCR method in patients with early-onset Parkinson disease from Russia

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Parkinson disease (PD) is the second most common neurodegenerative disorder. Increasing evidence suggests that genetic factors play an important role in the pathogenesis of Parkinson disease (PD). To date, genetic analyses have detected linkage to eleven chromosomal regions and have identified seven causative genes. Early-onset Parkinson disease (EOPD) may be associated with different mutations in PARK2 gene, from point mutations to complex rearrangements including deletion and/or multiplication of complete exons. Exon rearrangements occur with the rate from 33% to 66% of the total amount of mutations in PARK2 gene. It is therefore a question of scientific interest and necessity to develop a new cost-efficient, sensitive and fast method of detecting exon rearrangements. In the present study, we applied TaqMan Real-time PCR method to analyze and assess the frequency of deletions and duplications in PARK2 gene. We selected primers and probes for exons 2-12 and examined 63 EOPD patients from Russia (age of onset less than 50 years) for exon deletions and duplications. Exon rearrangements were detected in 14% of the EOPD patients in exons 2-7, being most frequent in exons 3 and 4. The results of our work let us presume that this method can be applied to mass screening of deletions and duplications in PARK2 gene in patients with Parkinson disease.

P0782. PARK2 mutations in patients with early-onset Parkinson's disease in Russia.

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Parkinson's disease (PD) is a progressive neurodegenerative disease with unknown ethiology and a complex interaction of genetic and enviromental factors. Mutations in the parkin gene (PARK2) have been identified as a relatively common cause of early-onset PD (EOPD) (age of onset before 50), especially in cases with a positive family history with an autosomal recessive mode of transmission. The aim of the present work was to identify mutations in the PARK2 gene in Russian EOPD patients (ethnic Slavic) and to study genotype-phenotype correlations. Using multiplex PCR, SSCP analysis, sequencing and quantitative real-time PCR with TaqMan probes we searched for PARK2 mutations in 40 patients with EOPD (age of onset 45±6.2 years). Polymorphism S167N have been identified in two patients and different mutations A man with heterozygous A334C substitution in functional region of parkin was characterized by early onset of disease and by weak response to L-dopa treatment. A woman with disease onset at 44 years has exons 8,9 deletion, disease progression was fast with good response to L-dopa. The deletions of exons 7, 10 and delition of exon 9 were found in two women with slow PD progression and onset of disease 45 and 50 years, respectively. The allelic localization of described mutations remains to be determined. The frequency of PARK2 mutation carriers is 10% with a wide variation in the clinical PD presentation.

P0783. Investigation of polymorphisms in coding region of Human Mitochondrial DNA in 20 Persian patients with Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder clinically characterized by bradykinesia, rigidity and tremor. The etiology of idiopathic PD is currently undefined and most PD cases are sporadic. Numerous findings have contributed to identifying possible mitochondrial involvement in the pathogenesis of PD. In order to identify polymorphic sites and potential genetic background accounting for PD and to test the hypothesis that mtDNA variations contributes to PD expression in Persian population, the complete region of ND1, $tRNA^{Leu}$, ND2 and 16s rRNA of mtDNA from 20 unrelated PD patients were evaluated. The sequences were aligned upon the revised Cambridge Reference Sequence (rCRS) and any incompatibilities were recorded single base substitutions (SBS), insertions and deletions (Indels). 9 polymorphisms were identified in this study which had not been already reported in the mitochondrial databases. Our study suggests that mtDNA mutations may corroborate the idea that the mitochondrial oxidative phosphorylation pathway is involved in the susceptibility to idiopathic PD and could contribute, together with nuclear gene mutations and environmental factors, to the pathogenesis of PD.

P0784. Functional analysis of pelota during the cell cycle

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Mutation in either the *Drosophila* pelota (*Pelo*) or the *S. cerevisiae* homologe, *dom34* cause defects of spermatogenesis and oogenesis in *Drosophila*, and delay of growth and failure of sporulation in yeast. Both phenotypes suggest a requirement of Pelo for normal progression of the mitotic and meiotic cell cycle. To explore the function of Pelo in mammals, we have disrupted the mouse *Pelo* gene and shown that the gene is essential for mouse embryonic development. Development of homozygous embryos arrests about 6.5-7.5 days after conception. The failure of ICM and survival of trophoblast in $Pelo^{-/-}$ blastocysts indicate that the lethality of *Pelo* is due to defect cell proliferation. Increase of polyploidy at E7.5 can be directly responsible for the arrested development and suggests that Pelo is required for the maintenance of the genomic stability. Approaches to establish Pelo-/-cells failed to detect a Pelo deficient cells. These results suggest that Pelo is essential for cell viability and cellular proliferation. Using Pelo specific antibody, we found that Pelo is associated with cytoskeleton. Western blot analysis revealed the presence of Pelo is in the cytoskeleton and membrane-fractions but not in nuclear and cytoplasmic fractions. These results demonstrate a possible role of Pelo in cytoskeleton organization and cell motility. Using yeast-two hybrid system, we identified several putative interaction partners of Pelota which are associated with the cytoskeleton. To overcome the early embryonic lethality of the Pelo deficient mice, generation of conditional knock-out mice is underway.

P0785. Distribution of Bgl II alleles at the phenylalanine hydroxylase gene in Republic of Moldova

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Phenylketonuria (PKU) is an autosomal recessive genetic disorder caused by phenylalanine hydroxylase (PAH) deficiency. The structural gene, located at chromosomal band 12q22-24, comprises 13 exons spread over 90kb of genomic DNA. The complete 2,4kb cDNA (Kwok, 1985) can be used to detect eight different RFLPs located within the PAH locus.

Genomic DNA was extracted and examined by standard procedures from 59 families with classical PKU, i.e. 236 parental chromosomes. PCR amplification of 290 bp fragment near from 1 exon and RFLP-analysis were performed as described previously by Dworniczak (1991).

Frequencies of normal alleles (see Table) slightly differ from average date in European populations, but not significant (χ^2 =0,05; p>0,8) and reveals more similarly with Germany and France (0,676/0,324). The level of observed heterozygosity in population of Moldova is 0,33, but the average date in European countries is 0,37. The distribution of mutant PKU alleles in our study not differs significantly from those observed in European (χ^2=0,20; p>0,08) and Asian (χ^2=0,95; p>0,08) populations. The Bgl II alleles in our populations had a significant difference in the distribution among normal and mutant chromosomes (χ^2=9,22; p<0,01). Frequency of informative cases by RFLP-analysis of Bgl II alleles from PKU families is 28% that provide a tool for molecular diagnosis of these disease and carrier status in Republic of Moldova.

Table. Distributions of Bgl II alleles at the PAH gene

Allele	Normal	Mutant	χ^2
E_1	0,716±0,038	0,284±0,038	9,22; p<0,01
E_2	0, 873±0,030	0,127±0,030	

P0786. Cellular responses to PHOX2B polyalanine aggregates associated with congenital central hypoventilation syndrome

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Congenital central hypoventilation syndrome (CCHS) is a neurocristopathy characterized by absence of adequate control of breathing, especially during sleep, with decreased sensitivity to hypoxia and hypercapnia. Mutations in the *PHOX2B* gene, encoding for a paired box homeodomain transcription factor required for the correct development of the autonomic nervous system, have been associated with the vast majority of CCHS patients. In particular, in a set of 60 CCHS patients we have identified 91,7% PHOX2B mutations, including 49 alanine expansions, 5 frameshift mutations and one truncated protein. The correlation between length of the alanine expanded tracts and severity of CCHS respiratory phenotype has been confirmed by length-dependent decrease in the transcriptional activation of PHOX2B target genes and length-dependent cytoplasmic PHOX2B retention with formation of aggregates.

In order to understand further the molecular mechanisms underlying the effects of PHOX2B polyalanine expansions, we have set up experiments aimed at assessing the fate of cells characterized by PHOX2B polyalanine aggregates. In particular, we have observed that the activation of heat shock response by the drug geldanamycin is efficient both in preventing formation and in disassembling PHOX2B polyalanine aggregates in COS-7 cells expressing PHOX2B-GFP fused proteins. Moreover, we have observed that inhibitors of the ubiquitin-proteasome pathway and autophagy, two mechanisms responsible for the maintenance of protein balance, increase percentage of cells characterized by PHOX2B polyalanine aggregates, suggesting their involvement in degradation of PHOX2B misfolded proteins. Finally, as proteins carrying polyalanine expanded tracts are prone to cell death, we are investigating toxicity due to mutant PHOX2B aggregates.

P0787. Mutations in the non-duplicated region of the PKD1 gene in families with autosomal dominant polycystic kidney disease in Czech Republic

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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 (14 % of ADPKD patients) genes, though in several ADPKD families the PKD1 and/or PKD2 linkage was not found.

PKD1 locus (MIM 601313) was linked to the short arm of chromosome 16, at 16p13.3 and so far 266 different germline mutations have been reported. PKD2 locus (MIM 173910) was localized to 4q13-23 and

73 different germline mutations were identified. Patients with PKD 2 mutation have milder clinical course in comparison with PKD1 patients.
The direct detection of mutations in the non-duplicated region of the PKD1 gene was performed in 90 nonrelated individuals ; in 32 families the disease was clearly linked to PKD1 gene and in 58 patients with end stage renal failure earlier than in 50 years. An affected member from each family was analyzed using denaturing gradient gel electrophoresis (DGGE). Samples which exhibited shifted bands on DGGE were sequenced in both directions.. We detected 19 mutations in 21 families/individuals; 16 mutations unique for Czech population. We identified 8 nonsense mutations, 6 missense mutations, 2 frameshifting mutations and 3 mutations in splice site.
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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P0788. MLPA analysis for the detection of exonic deletions in the PAH gene causing phenylketonuria

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Multiplex ligation probe amplification (MLPA) is a novel, sensitive and time and cost efficient strategy for molecular diagnosis of diseases involving deletions or duplications of large genomic regions. In phenylketonuria (PKU), most of the mutant alleles correspond to missense mutations, and large deletions have been scarcely identified. In this study we report for the first time the use of MLPA analysis in PKU patients to detect exonic deletions. A total of 24 PKU patients with an incomplete genetic diagnosis were subjected to MLPA analysis. The technique identified two different large genomic deletions in the phenylalanine hydroxylase (PAH) gene, of 6,6 Kb and 1,9 Kb and including exons 3 and 5, respectively, which were not detected by standard mutation analysis methods. The chromosomal breakpoints were established by long-range PCR and chromosomal walking, corresponding to mutations c.169-4951del6604ins8 and c.442-1556del1881, and confirming the involvement of repetitive sequences in each deletion (Alu sequences and simple repeats, respectively), as reported for other genomic deletions causing disease. The results show that MLPA can complement routine mutation screening in PKU patients, although, in the sample studied, exonic deletions in the PAH gene do not appear to be a frequent cause of PKU.

P0789. Association of the gene polymorphism of platelet glycoprotein ibα in acute myocardial infarction

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Thrombosis at the site of atherosclerotic plaque rupture is a prominent feature in acute coronary syndromes and in vessel wall injury after coronary intervention. The glycoprotein (GP) Ib-IX-V receptor complex contains 4 polypeptides, GP Ibα, GPIbβ, GPIX, and GPV. GP Ibα, which is the largest one, plays a crucial role in this process by mediating platelet adhesion by binding von willebrand factor at the site of the vessel wall lesion. We studied 540 subjects; 378 patients who underwent coronary angiography had myocardial infarction (MI) and 162 were healthy normal subjects based on noninvasive tests. We genotyped to determine the association of the -5T/C polymorphism of the platelet glycoprotein *GPIbα* with the potential risk factor for MI. In this study, we present the association of clinical findings and genotypes of the patients compared with the control data. The present findings showed no significant association between the genotype of the platelet glycoprotein *GPIbα* gene and MI, but evaluation of the *GPIbα* genotype might be valuable in clinical condition of patients with atherosclerotic vascular disease and MI.

P0790. Revisiting the mutational model leading to polyalanine repeat expansions and contractions

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Alanine stretches (AS) are common in all proteomes studied with the longer stretches being found in mammals. AS are coded by degenerated codons (GCN) and characterized by rapidly evolving nucleotidic sequences with high rates of expansions and contractions: the longer and more pure the sequence, the higher the length polymorphism of the AS in the population. These genic sequences are frequent in transcription factors and have recently been proposed as facilitators of evolution.
Hitherto, polyalanine expansions and contractions have been ascribed to 9 human diseases, either autosomal dominant or X-linked. Considering both that : i) AS are coded by mixed (GCN)n codons and, ii) that transmission over generations is stable, a polymerase slippage mechanism is unlikely. A mechanism of unequal allelic homologous recombination has thus been proposed. Worth noting, expansions have been identified in asymptomatic parents for 3/9 disease causing genes namely *HOXD13*, *ZIC2* and *PHOX2B* for which mutations account for synpolydactyly, holoprosencephaly and congenital central hypoventilation syndromes respectively. In the latest case, 5% of the asymptomatic parents from our series harbor an alanine expansion in their leucocytes. We tested carrier parents either by cloning the PCR fragment when a heterozygous SNP was present or by QMPSF. We show that, as speculated, carrier parents are somatic mosaics for the mutation. However, instead of the 3 alleles (wild-type, expansion and contraction) expected according to the mutational model, only 2 are observed in mosaic parents (wild-type and expansion). Therefore, an alternative mutational model to generate alanine expansions and contractions will be proposed.

P0791. Polycystin-2 regulates cellular proliferation in a p21/Cdk2-independent manner

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited disorders, characterized by progressive cyst formation and loss of kidney function. ADPKD is principally linked to two genes, PKD1 and PKD2. The pathogenesis of renal cyst formation is currently thought to involve dysregulated epithelial cell proliferation and differentiation, alteration in membrane proteins polarity and abnormal fluid accumulation. The molecular mechanism of cystogenesis originating from mutations in these two genes has not been fully elucidated. A recent report implicated polycystin-2 in the regulation of epithelial cell proliferation. Specifically, the authors suggested that PC2 over-expression suppresses cell proliferation through inhibition of the p21/Cdk2 pathway. To better understand the role of PC-2 in epithelial cell proliferation, we utilized various cellular models generated by stable expression of mutated (R742X and 1-702) and wild-type PKD2. In contrast to the previous data, over-expression of wild-type or mutated polycystin-2 in two different cell-lines does not affect cellular growth. Interestingly, primary renal epithelial cells from transgenic rats generated by expression of the 1-702 PKD2 have elevated levels of the proliferation markers, PCNA and c-myc. However, in both primary cells and stable cell lines, wild -type or mutated PC-2 do not alter p21 levels and Cdk2 activity. Collectively, these data suggest that in our models, PC-2 regulates epithelial cell proliferation in a p21/Cdk2-independent manner. In addition, these results highlight the fact that inactivation of PC-2 is probably not the only factor involved in the abnormal proliferation observed in cystic epithelial cells.

P0792. Polymorphisms of the catalase gene (CAT1) in the development of occupational chronic bronchitis

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Oxidative stress has been suggested to play an important role in the pathogenesis of occupational chronic bronchitis. Catalase together with other antioxidant enzymes constitutes a primary defense against oxidative stress. In this study, we investigate the possible association

of the catalase (CAT 1) 1167 T/C and -262C/T polymorphisms in the development of occupation chronic bronchitis.
The study group consisted of 135 patients with occupation chronic bronchitis from Republic Bashkortostan, Russian Federation. In control, the DNA samples from 299 unrelated healthy individuals were tested. PCR-RLFP method was used to detect catalase alleles. Statistical analysis of the results was carried out with Statistica v. 6.0 program.
No significant difference has emerged from the comparison of either genotype or allele frequencies for the catalase gene 1167 T /C polymorphism. The frequency of C allele -262C/T polymorphism was significantly higher in patients (81.48% compared to control 75.0%, χ2=3.99, p=0.05). A single base substitution C/T at position -262 have been found characterized higher expression of the catalase. Thus, catalase C allele significantly increased the risk for developing occupational chronic bronchitis in exposed workers (OR=1.47, CI=1.01-2.14). In conclusion, the catalase -262C/T polymorphism, perhaps, have dramatic role in the pathogenesis of lung diseases, particularly occupational lung disorders.

P0793. The Importance of the Genes, Responsible for Xenobiotics' Biotransformation in the Development of Reproductive Pathology in Women

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There was held an analysis of polymorphism of the genes responsible for xenobiotics' transformation (CYP1A1, GSTM1, GSTT1 and GSTP1) in women with reproductive pathology (primary infertility, secondary infertility and usual miscarriage) and in the screening group. Among the patients there was detected a significant rise of the deletion frequency of GSTT1 gene, compared with the results of the screening group (35,9% and 19,5% respectively, χ^2=9,77; p<0,003; OR=2,3). In the group of patients with a secondary infertility the deletion frequency of GSTT1 gene was 38,2%, while in the patients with an infertility after medical abortion it was 43,8%. In women with a secondary infertility the frequency of a mutant allele of the GSTP1 gene was 11,8%, and this was much higher than the control level, which was equal to 3,4% (χ^2=4,36; p<0,04; OR=3,92).

P0794. Pompe disease

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Glycogen storage disease type 2, also known as Pompe disease or acid maltase deficiency (AMD), is genetically transmitted through autosomal recessive inheritance. It is caused by a deficiency of acid a-D- glucosidase, a glycogen-degrading lysosomal enzyme. The enzyme catalyses complete hydrolysis of glycogen by cleaving a-1 , 4 and a 1, 6 glycosidic linkages at acid PH liberating glucose to cytoplasm for reutilization . The loss or diminution of GAA activity results in lysosomal glycogen accumulation in almost all body tissues with cardiac and skeletal muscle affected.Based on clinical symptoms and the age of onset, such defects divided into 3 clinical types: infantile, juvenile and adult forms. Muscle weakness is a prominent feature in all forms. Although in the infantile form, the rapid build up of the glycogen in muscle tissues causing sever muscle weakness, hypotonia, cardiomegaly, respiratory involvement, difficulty feeding and death during the 1st or 2nd age of old, in late-onset disease progression and death usually results from respiratory failure.Pompe disease is caused by mutation in the (GAA) gene. The GAA gene is located on chromosome 17q25.2-q25.3 .
Prenatal Diagnosis :determination of GAA in cultured amniotic cells or CVS,
clinical tests:Enzyme activity test,Enzyme level (ck,Ast,Alt) , (EMG), X-Ray, (ECG) ,Iscmic forearms test

P0795. Association of CYP1A2 and CYP2E1 polymorphisms and susceptibility to overt Porphyria Cutanea Tarda (PCT)

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Porphyria cutanea tarda (PCT) is characterized by partial deficient activity of hepatic uroporphyrinogen decarboxylase enzyme (UROD) with hepatic and cutaneous manifestations. The enzymatic defect of the UROD alone is not sufficient to provoke clinical symptoms of PCT. Various factors such as xenobiotics, alcohol, hormones and hepatitis infections are known to induce PCT. Cytochrome P450 enzymes are known to be involved in the metabolism of porphyrogens and therefore might have an important role on the pathogenesis of PCT.
The aim of the study is to determine the frequency of the CYP1A2 C/A polymorphism in intron 1 and polymorphisms in promoter (C1/C2) and intron 6 (D/C and 1/2) regions of the CYP2E1 gene.
The 117 PCT patients studied were divided into Familial-PCT, which are associated with mutations in the UROD gene and decrease erythrocyte UROD activity, and sporadic-PCT who exhibited normal UROD activity in erythrocytes. The rare familial occurrence of sporadic-PCT was classified as Type III-PCT. For the study PCR-RFLP analysis were carried out for the patients and 150 healthy volunteers.
Preliminary results support the idea that the "A" CYP1A2 allele could be a susceptibility factor for the development of PCT.

P0796. PPARα induces cardiomyogenesis during differentiation of mouse ES cells by utilization of a Reactive Oxygen Species (ROS) dependent mechanism

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Peroxisome proliferator-activated receptors (PPARα, -γ and -δ) are nuclear receptors involved in transcriptional regulations of lipid metabolism. The aim of this study was to investigate the role of PPARα in cardiomyogenesis during the differentiation of mouse embryonic stem (ES) cells derived embryoid bodies (EBs). When EBs were treated with PPARα agonists (WY14643, GW7647 and Ciprofibrate) a significant increase in cardiomyogenesis was observed. In contrast, the PPARα antagonist ,MK886, decreased the number of beating foci. The effect of the PPARα agonists was abolished when EBs were pre-incubated with the free radical scavengers Vitamin E (trolox) and N-(2-mercapto-propionyl)-glycine, indicating the involvement of reactive oxygen species (ROS). Furthermore, we observed an increase in ROS when EBs were treated with PPARα agonists, and consequently a decrease in intracellular ROS when EBs were treated with MK886. The effect of PPARα agonists on intracellular ROS was attenuated by NADPH-oxidase inhibitor DPI, indicating the involvement of NADPH oxidase. In summary our data indicate, that PPARα stimulation induces cardiomyogenesis in ES cells using a pathway that involves ROS and NADPH oxidase.

P0797. Analysis of a group of probands with Prader-Willi and Angelman syndromes by the MS-MLPA method

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Prader-Willi and Angelman syndromes (PWS and AS) are two distinct neurogenetic disorders caused by loss of function of imprinted genes in 15q11-q13. The loss of expression of these genes results from various mechanisms: deletion of the critical region, uniparental disomy, imprinting defects or gene mutation.
First, we studied 27 patients with PWS and 17 patients with AS, previously confirmed by microsatellite, FISH and methylation analysis by a new method, Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA). In the PWS group we confirmed all 19 deletions, 5 uniparental maternal heterodisomies, 2 uniparental maternal isodisomies, and one case with an imprinting defect. In the group of 17 AS patients we confirmed all 16 deletions and one uniparental paternal isodisomy.
Next we studied using MS-MLPA 5 additional new patients, 4 PWS and 1 AS, and we revealed 2 cases with uniparental maternal disomies, 2 cases with uniparental maternal isodisomies, and 1 case with uniparental paternal isodisomy.
We also applied the MS-MLPA method to two cases with marker chromosome 15 and an Angelman-like phenotype. We detected three alleles, two methylated and one unmethylated, in both cases.
The new MS-MLPA method is robust and highly useful for the detection

of copy number changes and methylation status in the critical PWS/AS region. The method is helpful and sensitive to distinguish between deletions and uniparental disomy or imprinting defects in suspected PWS/AS probands, and also to detect supernumerary marker chromosomes in the 15q11.1-q12 region.
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P0798. RPGR mutation in the patient with overlapping RP and PCD symptoms disrupts transport of the inner dynein arm component into the cilium

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Retinitis pigmentosa GTPase regulator (RPGR) protein has been localized to photoreceptors connecting cilia of rods and cones and in the transitional zone of cilia. Mutations in the *RPGR* gene on Xp21.1, responsible for 70% of the X-linked retinitis pigmentosa (RP), have been reported to be associated with retinitis pigmentosa, hearing loss, and bronchosinusitis. Recently, a mutation in *RPGR* has been reported in a family with overlapping phenotypes of the X-linked RP and primary ciliary dyskinesia (PCD). Here, we analyzed the coding sequence and intron/exon boundaries of the *RPGR* in a large Polish RP/PCD family. A missense mutation (G52R) found in the last nucleotide of *RPGR* exon 2 is an example of an exonic mutation disrupting the splicing process and leading to exon skipping. Immunostaining with fluorescent detection was performed on epithelial cells from nasal brushing. Predictably, RPGR was not present in the transitional zone of cilia in the patient. Another antibody, targeting a component of inner dynein arms, revealed defect of those structures in patient's cilia. These results indicate that RPGR plays an important role in the transport of inner dynein arms components into the cilium. Defect of this process may cause overlapping symptoms of X-linked RP and PCD.

P0799. Molecular analysis of the CPY1B1 gene in primary congenital glaucoma in a sample of Mexican patients

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Primary congenital glaucoma (PCG), an autosomal recessive disorder due to abnormal development of the anterior eye portion, is an important cause of childhood blindness. The principal molecular defect in most of PCG subjects occurs in the CYP1B1 gene, which is also expressed in the anterior chamber angle of the eye. CYP1B1 enzyme is able to metabolize steroid hormones and participates in tissue development. In the present study, we analyze the CYP1B1 gene of 25 non-related and sporadic and/or familial cases with PCG. Onset of clinical symptoms ranged from birth to 12 months (mean 4.5 months), the male:female ratio was 10:4 while ocular findings were similar in all patients except for two cases of difficult control. Consanguinity was observed in 3 families. DNA sequencing analysis of the CYP1B1 gene showed no missense or nonsense mutations in 21 cases, only polymorphic changes similar to those observed in normal controls were found. We observed four novel mutations in the rest of cases. These data allow conclude that most cases with PCG are not consequence of mutations in the CYP1B1 gene in our population, at least in the analyzed sample. This analysis is very important in diagnosis and genetic counseling of PCG in this population.

P0800. Structural and functional consequences of germline and somatic PTPN11 mutations on SHP-2 function

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Mutations in the protein tyrosine phosphatase PTPN11/SHP-2 are implicated in different human diseases, causing developmental disorders (Noonan syndrome, NS; LEOPARD syndrome, LS) or contributing to leukemogenesis, depending on the specific amino acid substitution. On the basis of previously gathered genetic and biochemical data, we proposed a model that splits NS- and leukemia-associated PTPN11 mutations into two major classes of activating lesions with differential perturbing effects on development and hematopoiesis. To test this model, we characterized biochemically a panel of SHP-2 mutants recurring in NS (T42A, A72S, T73I, E76D, E139D, I282V, N308D and M504V) and leukemia (A72V and E76K), and performed molecular dynamic simulations to determine the structural effects of selected mutants (A72S, A72V, E76D and E76K). Our results demonstrate that NS-causative mutations have less potency for promoting SHP-2 gain-of-function than do leukemia-associated ones. Simulations provide, for the first time, direct evidence supporting the hypothesis that mutations leading to strong basal activation, as observed among the leukemia-associated mutants, perturb the interaction between the N-SH2 and PTP domains, and describe the molecular interactions leading to the displacement of the N-SH2 loop from the PTP active site. Biochemical data demonstrate that the recurrent LS-causing Y279C and T468M amino acid substitutions engender loss of SHP-2 catalytic activity, identifying a previously unrecognized behaviour for this class of missense mutations and suggesting that these mutants interfere with normal SHP-2 function by a dominant negative mechanism. Finally, a classification of mutations based on the predicted role of affected residues is presented.

P0801. Mutational analysis of LMNA and ZMPSTE24 in restrictive laminopathy

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Restrictive dermopathy (RD) is characterized by intrauterine growth retardation, tight and rigid skin with erosions, prominent superficial vasculature, and epidermal hyperkeratosis, as well as bone mineralization defects, arthrogryposis, characteristic facial features, preterm delivery and early neonatal death. As in Hutchinson-Gilford syndrome (HGPS) - a laminopathy caused by mutations in LMNA - unprocessed prelamin A can be detected in cells and tissues of RD patients. Thus RD was shown to be caused by mutations in LMNA or ZPMSTE24 (FACE1) coding for a zinc metalloprotease, which is involved in the post-translational processing of prelamin A to mature lamin A (Navaro et al. 2004). In the present study, we conducted a mutational analysis of LMNA and ZMPSTE24 in six unrelated RD families. No mutations were found in LMNA. But three pathogenic DNA changes could be identified in ZMPSTE24. One of the three mutations was the common previously described mutation c.1085-1086insT leading to a frame shift, which results in a non-functional truncated peptide p.I362fsX380 (Navarro et al. 2005). Two novel mutations c.209_210delAT and c.50delA were found in two unrelated patients, putatively resulting also in a frame shift with the consequence of non-functional and even more truncated ZMPSTE24 peptides p.S70fsX73 and p.S17fsX37. The novel mutation c.50delA was combined with the common mutation c.1085-1086insT as a compound heterozygote in a German family. The novel homozygous mutation c.209_210delAT occurred in a consanguineous Turkish family. In the four other unrelated German families, the homozygous mutation c.1085-1086insT led to RD.

P0802. Analysis of the role of the NR2E3 (Nuclear Receptor) gene in Retinal Dystrophies

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Mutations in the NR2E3 gene are associated with various retinal diseases: Enhanced S-cone Syndrome, Goldmann Favre Syndrome and clumped pigmentary retinal degeneration. The involvement of the Nr2e3 gene in non-syndromic ARRP without clumped pigmentation has been documented in only one family.
The purpose of this report was to evaluate the involvement of the NR2E3 gene in Spanish patients with different types of retinal dystrophies: 96 ARRP patients, 4 cases with a clinical diagnosis of

retinoschisis without mutations in the XLRS-1 gene and 3 patients with Goldmann Favre Syndrome.
The coding regions of the NR2E3 gene were scanned for mutations using SSCP and direct sequencing methods.
Fourteen sequence variants were identified. Of these changes, six were interpreted as mutations (absence in controls and segregation with the disease within the family). 4/6 changes are novel: One frameshift mutation (c.1034_1038del5bp) and three different amino acid changing variants, one of which is located in DNA binding domain (p.S44L) and the other 2 changes remaining within the ligand binding domain (p.G287S and p.K324R) in the NR2E3 protein. We have also identified the missense substitution (p.R311Q) and the single splice-site change (c.119-2 A>C) described previously.
We detected 3 rare variants that include two novel changes, one of which is the synonymous codon change (c.195 C>T or p.N65N) and the other change involves a deletion of three base pairs in the 3'UTR region. The c.245+8 C>T change reported previously as non-pathogenic was also found. Several polymorphic variants were observed in NR2E3 gene.

P0803. Mutation analysis of RHO gene in patients with nonsyndromic retinitis pigmentosa from Bashkortostan

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Retinitis pigmentosa (RP), the hereditary degenerative disease of the photoreceptor neurons of the retina, represents the most prevalent cause of registered blindness among those of working age in developed countries. The prevalence of RP in the US and Europe is approximately from 1/3500 to 1/4000. It can be inherited as an autosomal dominant, autosomal recessive, or X-linked recessive disorder. In the autosomal dominant form (adRP), which comprises about 25% of total cases, approximately 30% of families have mutations in the gene encoding the rod photoreceptor-specific protein rhodopsin. More than 90 different rhodopsin point mutations have been identified, affecting 1 in 3500, or an estimated 2 million people worldwide. The RHO gene maps to human chromosome 3q21, consists of 5 exons. We have carried out mutations screening of this gene by SSCP-metod with further direct sequencing. We analyzed 5 exons of RHO gene in 119 unrelated patients with RP and their relatives and 77 unaffected individuals from Bashkortostan. Patients were examined clinically and with visual function tests. We have identified sequence change IVS3+4c→t, and its frequency more in patients (0.36) than in controls (0.1). There were statistically significant differences in allele and genotype frequencies at this locus in affected patients with RP and in controls. So, according to our data, this polymorphism is likely to be pathogenic. Also we analyzed poly $(CA)_n$ polymorphism Mfd2CA of RHO gene and revealed 14 allelic variants. Alleles 116 and 132 are significantly more frequent in patients than in controls.

P0804. Different Rhodopsin mutations at the same amino acid position lead to distinct phenotypes in patients.

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Purpose: To characterize the functional and clinical consequences of a novel rhodopsin (RHO) mutation in comparison to a known pathogenic variant at the same amino acid position.
Methods: 38 retinitis pigmentosa (RP) patients were screened for mutations in *RHO* by direct sequencing. Patients were characterized clinically using visual acuity testing, slit lamp examination, fundoscopy, Goldmann perimetry, dark adaptometry, and ERG recordings (ISCEV standard). Structural analyses of the RHO protein were performed with the Swiss-Pdb Viewer program.
Results: We identified a novel *RHO* mutation (G90V) in a three generation Swiss family. No additional mutation was found in this family in four other genes associated with RP. The clinical picture is compatible with RP. Interestingly, a different amino acid substitution at the same position in *RHO,* G90D, leads to night blindness instead of RP by constitutive low-level activation of the phototransduction cascade (Sieving et al., 1995). To elucidate whether the different amino acid substitutions have specific effects on the 3D structure of RHO, we did *in silico* simulations of the wild type, G90V and G90D variants of RHO. The overlay of the three RHO structures showed different distortions of amino acid 90 in variant G90D and G90V. Furthermore, the aspartic acid side chain in the G90D shifts the opposing oxygen of Leucin 112.
Conclusion: To our knowledge, this is the first description of two different phenotypes associated with mutations at the same amino acid position in RHO. Our data suggest that even small structural changes in RHO influence the human phenotype.

P0805. *KCNH2*: a good candidate for the cardiac phenotype observed in Rett syndrome.

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Rett syndrome (RTT) is one of the most common genetic causes of mental retardation in girls. Patients may survive into adulthood, but their life expectancy is reduced and the incidence of sudden death is greater than in the general population. Possible causes include cardiovascular anomalies. *MECP2* mutations are found in up to 90% of classic RTT cases. Given that MeCP2 is a transcriptional repressor, we decided to study *MECP2* mutation consequences on gene expression. For this aim, we selected three classic RTT patients with early truncating mutations and balanced X-inactivation. We isolated RNA from lymphoblasts of patients and three healthy controls and we performed gene expression profiling experiments on the two pools of samples. We used cDNA microarrays containing 19.200 DNA spots from human cDNA clones. We identified 75 up-regulated and 81 down-regulated genes. Among the down-regulated genes, we focused our attention on *KCNH2*, encoding the voltage-gated potassium channel alfa-subunit underlying Ikr, a current essential for human ventricular repolarization. *KCNH2* mutations are found in patients with Long-QT syndrome, a heart disease associated with sudden death due to ventricular arrhythmias. Previously, our group and others observed that electrocardiographic evidence of cardiac repolarization abnormalities, such as prolongation of QT interval, is common in RTT individuals. All three patients involved in the array experiments show significantly longer QTc values. Considering these data, *KCNH2* seems to be a good candidate for RTT cardiac phenotype. Experiments are ongoing in order to identify possible correlations between the type of *MECP2* mutation and *KCNH2* expression levels.

P0806. The *MECP2* gene mutation screening in Rett syndrome patients from Croatia

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Rett syndrome is an X-linked dominant neurodevelopmental disorder almost exclusively affecting females and is usually sporadic. Mutations in *MECP2* gene have been found in more than 80% of females with typical features of Rett syndrome. In this study, we analyzed 15 sporadic cases of Rett syndrome. In 7 of 15 patients (47%), we detected pathogenic mutations in the coding parts of *MECP2* fourth exon. We found two missense (T158M, R133C), two nonsense (R168X, R270X), two frameshift mutations (P217fs and a double deletion of 28-bp at 1132-1159 and 10-bp at 1167-1176) and one in-frame deletion (L383_E392del10). According to our knowledge, the last two mutations have not been reported yet. We also detected one previously described polymorphism (S194S). In conclusion, these results show that fourth exon should be the first one analyzed because it harbors most of the known mutations. Moreover, mutation-negative cases should be further analyzed for gross rearrangements. This is the first study of this kind in Croatia and it enabled us to give the patients an early confirmation of Rett syndrome diagnosis.

P0807. Novel MECP2 mutations identified in patients from southern Italy with Rett syndrome

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The Rett syndrome, a childhood neurodevelopmental disorder almost exclusively affecting females, is caused by mutations in the methyl-CpG-binding protein 2 gene (MECP2) located at Xq28. Previous studies carried out in patients coming from southern Italy divided into classical Rett, variant RTT and patients with Rett-like features, revealed mutations in MECP2 in most of the patients with classical and variant RTT, 73% of all the mutations were common mutations and other 27% were rare mutations (Conforti et al, Am J Med Genet A.,2003). By the additional studies to confirm the diagnosis of RTT, we report here two novel MECP2 mutations (N126Y and S134P) responsible for classical RTT. The missense mutation N126Y involves a well conserved aminoacid residue in MECP2 across species. It is considered to be associated with the clinical phenotype, since it is located at the methyl-binding domain and other pathogenic mutations were detected around the site. The second new mutation S134P is a missense mutation resulting from a nucleotide substitution of T to C; furthermore, it is a de novo mutation involving a conserved residue in the MBD of MECP2 gene. In addition to determine the role of X Chromosome Inactivation (XCI) in phenotypic variability of Rett patients, we also evaluated XCI using the AR methylation assay. Our data indicate that a random and a skewed XCI (90%) occurred in the patients carrying the S134P and the N126Y mutations respectively.

P0808. MECP2 mutation detection in Rett patients from Czech and Slovak Republics

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Background: Rett syndrome (RTT), primarily affecting females, is an X-linked neurodovelopmental disorder with a frequency of 1:10,000-1:15,000. RTT is caused by mutations in the MECP2 gene encoding methyl-CpG-binding protein 2. So far, a wide variety of mutation types have been reported including missense, non-sense mutations, small deletions/insertions and large rearrangements of the MECP2 gene. We present the mutation screening of the MECP2 gene in 92 girls with RTT from Czech and Slovak Republics.
Methods: Genomic DNA was extracted from peripheral blood leukocytes. The whole coding sequence and exon/intron borders of the MECP2 gene were amplified and analyzed by direct sequencing and RFLP. ,,Mutation-free" cases were examined by multiple ligation-dependent probe amplification (MLPA).
Results and discussion: Mutation analysis revealed 27 different point mutations and small deletions/insertions and 2 large deletions in 63 patients (68.4%). 33 patients had missense mutation, 17 patients had nonsense mutation, 9 carried frame-shift mutation (including 6 deletions, 1 insertion and 1 insertion/deletion) and 1 had in frame deletion. MLPA analysis revealed the deletion of exon 3 and 5`end of exon 4 in one patient and the deletion of exon 4 near STOP codon in another patient. Our results confirm the high frequency of MECP2 mutations in females with RTT and provide data concerning the mutation heterogeneity in the Slavonic population.
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P0809. Use of a microdissection laser system to study the possible involvement of some genes in the ring chromosome 20 epilepsy syndrome

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Ring chromosome 20 (r(20)) is a rare chromosomal anomaly always associated with typical and intractable epilepsy. This epilepsy syndrome is characterized by a ring chromosome mosaicism : the percentage of cells carrying r(20) varied in a cell line. Currently, no large deletions on the ring chromosome 20 have been found by cytogenetics. The aim of this work is to reveal the possible involvement of some genes located on the chromosome 20 in this syndrome. The genes KCNQ2 (potassium voltage-gated channel KQT-like protein 2) and CHRNA4 (neuronal nicotinic acethycholine receptor alpha4 subunit) both involved in other form of epilepsy are two serious candidate genes. A mutation or a deletion in these two genes on the r(20) could explain the phenotype of epilepsy. To undertake this study, we need to release from the mosaicism. A microdissection laser system was used to isolate the ring chromosome. For a patient, about twenty r(20) were collected in the same tube. Next, whole genome amplification followed by specific polymerase chain reactions (PCR) on the candidate genes were performed. After having demonstrated that the sample contained only the r(20), using microsatellite markers, the two genes were sequenced. So, this technique of microdissection can be a solution to study other pathologies associated with a mosaicism.

P0810. Molecular Investigation SCA in 26 patients suspected to SCA in Iran

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More than 20 types of SCA have been described. These types are given numbers (1-22, excluding the number 9).
All types of SCA are characterized by a progressive incoordination of walking. They are often associated with poor coordination of hand movments, eye movments, and speech. With some exceptions, the onset of symptoms usually occurs after the age of 18.SCA is slowly progressive M.R.I and C.T of affected persons often show shrinkage or atrophy of cerebellum.
Most common of SCA is SCA3 (21%), SCA2 and SCA6 (15%)
SCA 1 (6%) and SCA7 (5%) respectively.
The genetic change that causes SCA types 1,2,3,6,7,12 and 17 is called a CAG repeat expantion.
We checked these types of SCA in our lab with PCR analysis across the CAG region of the SCA1, SCA2, SCA3, SCA6, and SCA7 genes to determine allele sizes.
We checked 28patients refer to our lab for detection of these types of SCA by molecular technique. 13 patients were normal repeat size for these types of SCA and 4 patients were expanded repeat size (2 patients SCA3, 2 patients SCA62 and patients SCA2). 9 patients have intermediate repeat size. After detection of normal repeat, we checked other hereditary ataxia. Then, we proved AT for 2 patients by molecular technique.

Type	Chromosome	Normal repeat size	Expanded repeat size
SCA 1	6p23	6-36	39-83
SCA 2	12p24	15-31	34-220
SCA 3	14q24.3-q32	12-40	55-86
SCA 6	19	4-18	21-33
SCA 7	3p12-p21.1	4-19	37-300

P0811. Marked genetic anticipation in Spinocerebellar Ataxia 2 and Huntington Disease. New insights on phenotypic variability in autosomal dominant neurodegenerative diseases

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CAG expansion is the causative mutation in a heterogeneous group of hereditary neurodegenerative disorders. It is widely reported that the size of the repeats is inversely correlates with the disease age of onset. We present Egyptian families with SCA 2 and Huntington Disease, in whom marked genetic anticipation can not be only explained on the basis of CAG repeats number. In the SCA2 family, the proband, who is a boy is presented as early as 2 years of age with delayed milestones, upper extremities tremors and dystonic movement of limbs. Molecular analysis of his SCA2 gene revealed a CAG expansion of 75 repeats. His affected mother, who manifested at the age of 22, died before being examined. Neonatal onset of SCA2 has been reported before with 62 repeats, which is still lower than repeats detected in our proband. In one of our HD families, the amplified HD allele passed from the mother with a limited further expansion to one son only, out of her

four offsprings. The interesting feature in this maternal transmission is the marked anticipation in the age of onset with more than 10 years advance in appearance of symptoms in her son, in spite of the limited difference in HD allele size. We report here on the potential presence of certain brain related biomarkers that can modify expression and effect of mutated polyglutamine proteins and hence promote variability in disease age of onset, and mimics the situation currently proven in another age-related neurodegenerative disease; Parkinson's disease.

P0812. A Point Mutation AT the Calreticulin Gene Core Promoter conserved sequence in a Case of Schizophrenia

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Exposure to atypical antipsychotic drugs such as valproate increases the expression of chaperones that assist in the folding of proteins in the endoplasmic reticulum (ER) including calreticulin, GRP78/BiP, GRP94, and PD1. This neuroprotective role may be involved in the pathophysiology of neuropsychiatric disorders such as schizophrenia and bipolar disorder. The 5'-flanking region of the human calreticulin gene was screened in 100 cases of schizophrenia by PCR/SSCA between -485 and +1 basepair (bp) relative to the transcription start site. A G>C point mutation was detected at -48 in a case of paranoid schizophrenia, which was not detected in 280 unrelated control subjects (560 chromosomes). This is the first report of mutation in relation with the calreticulin gene. The -48G>C mutation creates a CpG site at the core promoter region. The role of this mutation remains to be clarified in the pathophysiology of schizophrenia.

P0813. Identification of X linked gene for Severe Combined Immunodeficiency in irainain patients

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Severe combined immunodeficiency (SCID) is a syndrome of profoundly impaired cellular and humoral immunity. In humans, SCID is most commonly caused by mutations in the X-linked gene IL2RG, which encodes the common chain, c, of the leukocyte receptors for interleukin-2 and and multiple other cytokine receptors, including those for IL-4, IL-7, IL-9, and IL-15. Without bone marrow transplantation (BMT), affected patients suffer severe and persistent infections, often with opportunistic pathogens, and generally die in infancy. Although both X-linked recessive and autosomal forms of SCID are recognized, the X-linked form is the most frequent. Patients with X-linked SCID generally have very low numbers of T cells and natural killer (NK) cells, whereas B cells are often found in relatively high numbers even though specific antibody responses are deficient

We want to investigate the frequency and variety of X-linked that cause SCID in Iranian patient that no one work on it and we want to analyze Exon 3,4,5 and find the mutation.according to that we can design prenatal diagnosis kit.

P0814. Succinate Dehydrogenase and Citrate synthase gene expression in idiopathic infertility of testicular origin

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Sperm cell concentration in the ejaculate has been correlated with the nuclear-encoded [Succinate Dehydrogenase (SDH)/ complex II and citrate synthase (CS)] mitochondrial enzyme activities (Ruiz-Pesini et al., 2000). From these data, we postulate that the enzymatic expression should be decreased in severe sperm impairment and investigate if this decrease could be a consequence of gene expression variation.

By means of a quantitative real-time PCR we have determined the expression pattern of *SDHB*, *SDHC*, *SDHD* and *CS* gene in testicular biopsies of 14 infertile men with a phenotype of non-obstructive azoospermia or severe oligozoospermia (<5 million sperm per ml) and 13 infertile men with obstructive azoospermia as controls. The RT-PCR reactions were performed in a LightCycler® Instrument (Roche) using SYBR Green I fluorescence dye. Housekeeping glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ciclophilin-A and β2-microglobulin (β2M) genes were used as endogenous control genes to normalize the results. The statistical analysis was performed using the relative expression software tool (REST©).

High real-time PCR efficiencies were obtained for the seven genes analysed, between 1.75 and 1.92. The results obtained after normalization with GAPDH expression data agree with those obtained with ciclophilin-A but not with β2M, suggesting that the β2M gene is not suitable for normalization of the target genes data. Our preliminary results showed no statistical difference in gene expression of the four genes analysed between groups suggesting that the expression of these nuclear-encoded mitochondrial enzymes are not transcriptionally regulated in severe sperm impairment.

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P0815. Study of the Spatial Pattern of Expression of the *Spp2* Gene in Mouse Liver Using a Non-Isotopic *In Situ* Hybridisation Method

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Secreted phosphoprotein 24 (spp24) is a member of cystatin superfamily and was first identified in cattle as a minor component of cortical bone. Subsequently it was identified as a component of the fetuin-mineral complex. In the original study, using northern blot analysis, the expression of the gene (*Spp2*) was demonstrated in bovine bone periosteum and liver. We assessed the pattern of expression of spp24 in human and mouse tissues. In human, using hybridisation of a full-length spp24 cDNA probe to a human multiple tissue expression (MTE) mRNA array, the strongest hybridisation was identified in adult liver and kidney. In mouse, using an RT-PCR method, expression was detected in liver, kidney, brain and diaphragm. Because the gene is expressed at the highest level in all three species in liver, it was decided to assess its spatial expression in mouse liver using a non-isotopic *in situ* hybridisation method. The expression of *spp2* in mouse liver was demonstrated most strongly in:

• Hepatocytes across the liver, but with prominent expression especially adjacent to vessels around the portal vein.
• Endothelial cells.

In addition lower levels of hybridisation suggestive of expression of spp24 were detected in:

• Artery smooth muscle cells.
• Connective tissues around the vessels, especially around the portal vein.

The gene is not detectably expressed in polymorphonuclear cells (neutrophils) that were present in the tissue sections.

These findings could help us understand the function of spp24 protein.

P0816. Characteristation of mouse Dactylaplasia mutations, a model for human ectrodactyly SHFM3

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SHFM3 is a congenital limb malformation affecting the hands and feet. It is caused by an ~500kb duplication at 10q24. Subsequent work from our laboratory narrowed the minimal duplication to a 325kb segment containing two genes. *Dactylaplasia* (*Dac*) is a similar inherited limb malformation in mice and is thus a model for human SHFM3. Sidow *et al* reported two alleles, Dac^{1J} and Dac^{2J}, mapping in the region syntenic with the duplication in SHFM3. Dac^{1J} is an insertion of a transposon upstream of *Fbxw4* and was sequenced, while the exact molecular lesion in Dac^{2J} has not yet been characterized. Here, we report mapping and sequencing of Dac^{2j} by inverse PCR and show that it is caused by insertion of an early transposon, similar to the Dac^{1j} allele. Interestingly, this mutation occurs within a highly conserved element that may represent a regulatory sequence. The two *Dac* insertions are ~50kb apart. We tested for duplication of the region in

Dac mice, since this is the mutation mechanism in human SHFM3. Quantitative PCR on genomic DNA with assays covering the genomic locus failed to identify any copy number differences between either *Dac* mutant and wild-type littermates. Both the human and mouse phenotypes seem to be caused by a disruption in the normal gene expression patterning in limb bud development, but the mechanisms are not known. Complementary approaches in both human SHFM3 and mouse *Dac* will be important in identifying the causative genes and uncover the regulatory mechanisms involved in gene expression in this region.

P0817. Beta Globin gene cluster haplotypes among sickle cell patients and carriers in the Puerto Rican Population

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Sickle hemoglobin (HbS) is the most common hemoglobin variant worldwide and is associated with four major haplotypes on the β globin gene cluster: #19 (Benin), #20 (Bantu, Central African Republic (CAR), #3 Senegal and #17 (Cameroon). The CAR haplotype is associated with a more severe phenotype, while the Senegalese haplotype is not. Other factors, (gender, modifier genes, fetal hemoglobin (HbF) levels, etc are thought to influence sickle cell disease (SCD) severity. We studied 33 patients with homozygous HbS mutations to compare disease severity with β globin gene cluster haplotype. The majority of the patients were haplotype heterozygotes (19/20 42.4%, 19/3,31 18.2%), 21.2% were homozygous for the CAR haplotype and only 6.1% were homozygous for the Benin haplotype, which partially contradicts the historical records of the African slave trade. Patients homozygous for the CAR haplotype, 50% of the 19/20 haplotype heterozygotes and 33% of the 19/3,31 haplotype heterozygotes had severe manifestations of SCD. Clinical severity did not correlate with HbF levels and β globin haplotypes. Puerto Rican subjects carrying the sickle cell trait (AS, 79 parents and 81 infants) and 61 normal, the predominant haplotypes were the Benin and CAR haplotypes. The AA chromosomes possessed mutations commonly associated with S chromosomes which indicates admixing in the Puerto Rican population. Our results resemble the haplotype distribution reported for the island of Cuba, where the predominant haplotypes were the Benin and the CAR haplotypes and the Senegalese haplotype contributed less than 10% of the HbS chromosomes. Research supported by RCMI grant G12RR03051.

P0818. Frequency and etiology of uniparental disomy in Silver Russell Syndrome

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Silver-Russell syndrome (SRS) describes a uniform malformation syndrome characterized by pre- and postnatal growth restriction and a typical craniofacial feature. The basic defect of SRS is currently unknown, and the number of meaningful genetic tests available is therefore limited.. We have reported the etiology of uniparental disomy (UPD) in Silver-Russell syndrome (SRS) patients. Thirteen families were typed with 4 short tandem repeat markers from chromosomes 7. Maternal UPD7 was detected in one SRS patient, accounting for approximately 7% of the tested SRS patients. Our patient with UPD7 and those previously published had a classical SRS phenotype and were not clinically distinguishable from other children diagnosed with SRS. These results agree with previously published studies. The allelic distribution in the family with UPD showed complete heterodisomy that indicating UPD originates from maternal meiosis. Our results demonstrate the necessity of screening SRS patients for UPD7, although the effect of UPD7 cannot be correlated with the SRS phenotype as yet.

P0819. Founder mutation S133T (p.Ser133Thr) of the SLC26A4 gene in Turkish families with Pendred Syndrome

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The gene SLC26A4 encodes a chloride/iodide and chloride/formate transporter implicated in various forms of human hereditary hearing impairment.
Mutations of SLC26A4 were first identified in Pendred Syndrome by Everett in 1997, and have since been further associated with the non-syndromic autosomal form of deafness DFNB4 and with Enlarged Vestibular Aqueduct Syndrome.
We here report three unrelated non-consanguineous Pendred families from Turkey in which all patients presented with profound to severe deafness and goiter. Molecular analysis of the 20 coding exons of SLC26A4 with denaturing high-performance liquid chromatography (DHPLC) followed by sequencing elicited the same homozygous p.Ser133Thr (c.398T>A) mutation in all patients. This mutation affects an amino acid conserved in all vertebrate species and located in the SLC26A transporters signature domain. It was not observed in 100 chromosomes of normal-hearing individuals.
Since Borck (2003) reported the p.Ser133Thr mutation at the homozygous level in another Turkish family, and since all families analysed here originated from either east or south of Turkey, we set out to examine microsatellite and SNP markers linked to the SLC26A4 locus in 7q31.1 whether a founder effect or recurrent mutation might be at cause.
A single haplotype was shared by all patients analysed, suggesting that p.Ser133Thr is a founder mutation in Turkish patients with Pendred syndrome.

P0820. Strategy for the generation of a knockout mouse for LAT-2

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The *SLC7A8* gene encodes for LAT-2, a transporter of neutral amino acids that belongs to the heteromeric amino acid transporters (HAT) family, which is formed by a heavy subunit (rBAT or 4F2hc) linked by a disulfide bridge to a range of light subunits. Transporter 4F2hc/LAT-2 plays a major role in the net basolateral efflux of cysteine, pointing to LAT-2 as a candidate to modulate cystine reabsorption. Here we describe the generation of chimeras for a knockout of *Slc7a8*, that can contribute to ascertain the possible implication of LAT-2 in cystinuria and to study the physiological function in skeletal muscle and heart, where it has a remarkable expression.
We obtained the sequence of the murine gene in the Celera database, and designed primers in the 5' region of the gene to amplify the homology arms, from 129P2 genomic DNA. We generated a vector with homology arms of 6,1 kb and 2,3 kb, and replaced part of the promoter and exon 1 of *Slc7a8* by the neomycin resistance gene.
Homologous recombination between the vector and the *Slc7a8* gene in 129P2 ES cells was performed by GenOway (Lyon). We screened 400 clones by long PCR and confirmed by Southern-blot that 3 clones were heterozygous for the mutation in *Slc7a8*. Two clones were microinjected into C57BL/6 blastocysts, and several chimeric mice were obtained.
At present we are crossing chimeras with C57BL/6 females to obtain heterozygous mice.
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P0821. Maternal Alleles of Genes Involved in Cholesterol Transport are Modifiers of the Smith-Lemli-Opitz Syndrome

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The Smith-Lemli-Opitz Syndrome (SLOS,MIM 270400) is a malformation syndrome that ranges in clinical severity from minimal dysmorphisms and mild mental retardation to severe congenital anomalies and intrauterine death. SLOS is caused by mutations in

the delta7sterol-reductase gene (DHCR7), which impair endogenous cholesterol biosynthesis and make the growing embryo dependent on exogenous (maternal) sources of cholesterol. Previous studies demonstrated a correlation of severity with DHCR7 genotype and maternal ApoE genotype. We have now investigated whether other genes involved in lipid metabolism (apoCIII, LCAT, CETP, LDLR, ABCA1), and additionally MTHFR, involved in folic acid metabolism, may act as modifiers of the severity of SLOS. SNP genotyping was performed in 68 SLOS patients, their mothers and fathers.
We tested for correlation between patients´ clinical severity score and gene dose of the rare alleles in the patients and their parents. Neither patients nor paternal genotypes were associated with disease severity. In addition to the previously observed association with DHCR7 genotype and apoE genotypes, only maternal ABCA1 genotypes (p=0.007) but no SNPs in other genes showed a significant correlation. The rare maternal K1587 allele in the ABCA1 gene was associated with milder phenotypes. The correlation of maternal ABCA1 genotypes and SLOS severity persisted after stratification for cholesterol (p=0.041) and for the DHCR7 genotype of the patient (p=0.008).
ABCA1, a transporter of cholesterol across membranes is highly expressed in the placenta. We conclude that at least three factors, DHCR7-, ApoE- and ABCA1 genotypes modify the phenotype of the SLOS.
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P0822. SMN1 and SMN2 deletion of exons 7 and 8 concurrent in one family

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disorder that characterized by loss of α - motor neurons due to degeneration in the lower motor neurons . The SMN gene exists in two highly homologous copies, telomeric (SMN1) and centromeric (SMN2). SMA is caused by mutations in SMN1 but not SMN2.
In this study one family who were heterozygote for both SMN1 and SMN2 gene deletion screened for common deletion on exon 7 and 8 in both genes. They missed only their child because of SMA type I caused by deletion in exons 7 and 8 in SMN1 gene. Heterozygosity of parents was found when they referred to prenatal diagnosis in next pregnancy .PND showed deletion of exons 7 and 8 concurrent in SMN2 gene. It proved that parents were heterozygote for deletion of exons 7 and 8 in both genes SMN1 and SMN2. Mutations in SMN1 are responsible for SMA and mutations in SMN2 just influence on severity of SMA. Also, so far authors have not been observed any report about deletion of exons 7 and 8 together in SMN2 gene.
Genetic counseling could have opinion about SMA because of found intact SMN1 gene in PND, but we can not ignore the possible role of homozygous SMN2 deletion in exons 7 and 8 either in SMA or other disorder such ALS and CMD that SMN2 gene can act as a prognostic factor and may be a phenotypic modifier in sporadic ALS.

P0823. Molecular analysis of common mutations associated with Spinal Muscular Atrophy (SMA) in Romanian families

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of lower motor neurons leading to muscular weakness and atrophy. Any data regarding prevalence and carrier frequency in Romanian population are not available.
The aim of our study was to analyze the most common mutations that occur in SMN and NAIP genes, causing SMA disease, in Romanian families.
This is the first molecular test available for diagnosis of SMA in our country. Genetic counseling was provided to all the couples having a child affected by SMA.
We investigated 45 SMA clinically diagnosed patients from "Al. Obregia" Hospital Bucharest, and their 34 relatives. All patients fulfilled the diagnostic criteria of SMA as defined by International SMA Consortium. We screened our subjects for the homozygous deletion of SMN genes exons 7 and 8 and NAIP exon 5, using PCR-RFLP and PCR techniques.
The results are shown in Table:

Homozygous deletions	SMA I n = 26	SMA II n = 10	SMA III n = 9
SMN1 Exon 7	4 (15,4%)	1 (10%)	0 (0%)
SMN1 Exon 8	4 (15,4%)	1 (10%)	0 (0%)
NAIP Exon 5	0 (0%)	0 (0%)	0 (0%)
SMN1 Exons 7 + 8	8 (30,8%)	1 (10%)	1 (11%)
SMN1 Exons7 + 8 + NAIP Exon 5	5 (19,2%)	0 (0%)	0 (0%)
SMN1 Exon 7 + NAIP Exon 5	1 (3,9 %)	1 (10%)	0 (0%)
SMN1 Exon 8 + NAIP Exon 5	1 (3,9%)	0 (0%)	0 (0%)
None	3 (11,4%)	6 (60%)	8 (89%)

It is important to notice one homozygous deletion of SMN1 exons 7 and 8 among SMA III clinically diagnosed patient.
Additionally, we have identified in the relatives group, only three particular homozygous deletion: SMN2 exons 7+8, SMN2 exons 7+8+ NAIP exon5, and NAIP exon5. Interestingly, their SMA type II (n=2) and III (n=1) clinically suspected children have no homozygous deletions in these exons. Supplementary studies are required for establish the meaning of this findings.

P0824. A possible distortion in the segregation ratio of the SMN1 alleles in SMA families

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Spinal muscular atrophy (SMA) is a common autosomal recessive disorder caused by mutations in the SMN1 gene. Both parents of a patient are carriers with a recurrence risk of 1/4. Our objective was to analyse the segregation ratio of the SMN1 gene in the offspring of SMA parents in whom de novo mutations or mosaicism were ruled out. We present the results from 216 prenatal analyses and from 101 carrier studies in unaffected siblings.
A total of 46 foetuses (21%) were homozygously deleted (expected n=54), 91 (42%) were carriers (expected n=108) and 79 (36%) were homozygous for the wild type allele (expected n=54). The transmission rate of the deleted gene copy was lower than expected (p=0.001). Furthermore, from 101 unaffected siblings of patients, 57 (56%) were carriers (expected n=67) and 44 (43%) were non-carriers (expected n=33). Taking together the unaffected foetuses and siblings, the number of carriers was 148 (55%, expected n=179) and that of non-carriers was 123 (45%, expected n=89) (p=0.0001). No significant differences in the sex-ratio of the foetuses and carriers were observed. Nor were there any divergences in the paternal or maternal origin of the transmitted allele to the carriers.
These results suggest a distortion in the segregation ratio in favour of the wild type SMN1 allele. Meiotic drive or preferential survival of gametes may account for these findings. However, to determine their significance in SMA biology and their consequences in genetic counselling, larger population studies in SMA families are necessary.
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P0825. Polymorphisms of plasminogen activator inhibitor-1, angiotensin converting enzyme and coagulation factor XIII genes in patients with spontaneous abortion

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Introduction: Spontaneous abortion is a common problem among infertile couples. Thrombophilic disorders and hypofibrinolysis could result in abortion due to uteroplacental microvascular thrombosis and hypoperfusion. Genes that influence the coagulation are potential etiologic candidates for thrombophilia. We investigated the polymorphisms of plasminogen activator inhibitor-1(PAI-1), angiotensin converting enzyme (ACE) and coagulation factor XIII (FXIII) genes in Iranian women with spontaneous abortion.

Materials & Methods: 120 patients with more than two abortions and 112 healthy female controls without history of abortion were involved. In order to characterize PAI-1 (4G/5G), ACE (D/I) and FXIII (val 34 leu) polymorphisms, we performed polymerase chain reaction followed by digestion with restriction enzymes (PCR-RFLP).

Results: 16 (14.4%) patients were homozygote (4G/4G) for PAI-1 polymorphism, in contrast with two (2%) controls ($p = 0.001$). In patients with more than six abortions, 4G homozygosity was more frequent ($p < 0.05$). 38 (29.5 %) patients and 25 (26.6%) controls were homozygote (DD) for ACE polymorphism. DD homozygosity was more frequent in patients with more than seven abortions ($p < 0.05$). We observed only two patients and one control with homozygosity (34leu) for FXIII polymorphism.

Conclusion: 4G/4G polymorphism for PAI-1 gene could be a thrombophilic mutation leading to abortion. We recommend analysis of this mutation in patients with spontaneous abortion, especially if the history of several abortions exists.

P0826. Partial paternal uniparental disomy (UPD) of chromosome 1 in one patient with Stargardt disease

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Stargardt disease (STGD) is the most common juvenile macular dystrophy, characterised by central visual impairment. All recessively inherited cases are thought to be due to mutations in the ABCA4 gene.

A total of 77 STGD families were studied. DNA from every patient and relatives was analysed for variants on the ABCR400 microarray; results were confirmed by direct sequencing. Haplotype analyses, standard and high-resolution karyotypes and the novel approach of Multiplex Ligation-dependent Probe Amplification (MLPA) were also performed.

A patient with STGD caused by the homozygous R1129L mutation in the ABCA4 gene was found to be the daughter of a non carrier mother and a father who was heterozygous for this change. Haplotype analysis suggested that no maternal ABCA4 allele was transmitted to the patient. Microsatellite markers spanning all chromosome 1 could identify a homozygous region of at least 4.4 Mb, involving the ABCA4 gene. The cytogenetic study revealed normal female karyotype. Further evaluation with MLPA resulted useful for showing that the patient had a normal dose for both copies of the ABCA4 gene, thus suggesting partial paternal isodisomy but not a maternal microdeletion.

We report, for the first time, that recessive STGD can rarely be inherited from only one unaffected carrier parent in a nonmendelian manner. This study also demonstrates that genomic alterations contribute to only a small fraction of disease-associated alleles for ABCA4. Nevertheless, for those cases where homozygous mutations are found, is recommendable to perform haplotype analyses, in order to discard a possible situation of UPD.

P0827. Segregation analysis of the X-chromosome in patients with steroid sulfatase deficiency

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X-linked ichthyosis (XLI) is an inherited metabolic disease due to steroid sulfatase (STS) deficiency. Most XLI patients (>90%) have entire deletion of the STS gene and flanking markers. The presence of low copy number repeats (G1.3 and CRI-S232) on either side of the STS gene seems to be responsible of the high frequency of these deletions. Unequal homologous recombination between both X-chromosomes seems to generate this type of deletion. In the present study, we analyzed 6 Mexican families with XLI and complete deletion of the STS gene to investigate the parental origin of the affected X-chromosome. STS activity was measured in leukocytes using 7-[^{3}H]-dehydroepiandrosterone sulfate as a substrate. Amplification of the regions telomeric-DXS89, DXS996, DXS1139, DXS1130, 5' STS, 3' STS, DXS1131, DXS1133, DXS237, DXS1132, DXF22S1, DXS278, DXS1134-centromeric was performed through PCR. Segregation analysis was performed with GeneScan**.** No STS activity was detected in the XLI patients (0.00 pmoles/mg protein/h). Paternal origin of the affected X-chromosome was confirmed in two families. It was not possible to determine the parental origin of the X-chromosome in fourth families. All patients showed the 5' STS deletion at the sequence DXS1139 (probe CRI-S232A2) and the 3' STS rupture site at the sequence DXF22S1 (probe G1.3). These data indicate that unequal homologous recombination between both X-chromosomes is not the only mechanism in the genesis of the breakpoints of the STS gene of XLI.

P0828. Screening for inherited thrombophilia in a group of Greek patients with stroke.

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Stroke is a complex heterogeneous disease with unclear etiology. Inflammation, abnormal coagulation, metabolic diseases, environmental and nutritional factors including hypelipidemia have been greatly implicated in the pathogenesis of the disease. As a result, a wide spectrum of genes with different roles have been suggested to be associated with the disease. In the present study, we evaluated 16 patients with ischemic stroke and one patient with hemorrhagic stroke for the presence or not of common mutations and/or polymorphisms in Factor V Leiden (G1691A), Factor II (G20210A), MTHFR (C677T), GPIa (C807T) and PAI 4G/5G. Our results indicate that 11.7% of the patients were heterozygous for the FV mutation, 5.8% were heterozygous for the FII mutation, and 52.9% were heterozygous for the MTHFR polymorphism. Homozygosity for the tested genes was not detected in any patient, neither the stroke-associated 4G/4G genotype of the PAI gene. In our patients group, 11.7% were homozygous and 29.4% were heterozygous for the GPIa polymorphism.

Our results indicate that the Factor V Leiden mutation was more prevalent in patients with ischemic stroke (around 2x) compared to the carrier rate in the general population (5% of carriers rate). Factor V Leiden has been associated with venous thrombosis and there are studies suggesting its involvement in stroke, although there is still controversy in the field. Our findings indicate the necessity for larger, well controlled studies for the identification of the role of Factor V mutations in the pathogenesis of stroke.

P0829. The role of TBX1 in the 22q11DS phenotype

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Del22q11.2 syndrome (22q11DS) occurs in 1 of 4000 life births and is the most frequent chromosomal microdeletion found in men. 22q11DS is characterised by many and diverse clinical symptoms, all showing variable expressivity and incomplete penetrance. Most patients with the 22q11DS phenotype carry a 1.5-3 Mb-wide chromosomal

microdeletion on 22q11 (del22q11).
Chromosome engineering experiments in mice strongly suggest that the majority of the physical phenotype in 22q11DS results from haploinsufficiency of *TBX1* . Recently, evidence that haploinsufficiency of *TBX1* also plays a key role in the human phenotype was provided by the identification of point mutations in patients from 3 families with typical clinical features of 22q11DS, but without deletions .
Here we report a fourth family in which the phenotypic features of 22q11DS, in particular VCFS, segregate with a novel 23 bp-frameshift deletion within the 3'-end of *TBX1C* (1320-1342del23bp).
Based on CAT assays and immunocytochemical investigations of transfected cells we provide evidence that this mutation impairs the ability of TBX1 to activate transcription in reporter experiments although it localises in the nucleus.This corroborates earlier findings that the C-terminal region of Tbx1 is essential for transcriptional transactivation. Our results suggest that the novel 23 bp-frameshift deletion found in this family represents a null allele and is likely to cause TBX1 haploinsufficiency in non-deleted VCFS patients.

P0830. Pattern of β-globin mutations among β-thalassaemic children and families in Albanian population

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Objective. The aims of this study were to determine the different β-thal alleles that are present in Albanian population, to establish an efficient cost- effective method for molecular detection of the heterozygous carriers in the screening programmes and prenatal diagnosis of β-thalassemia .
Methods. We have analyzed 92 samples, of these 42 patients with thalassemia major or intermedia, 13 with sickle cell anemia or sickle cell β-thalassemia, 33 parents and 4 unrelated heterozygotes. The mutations were investigated by TTGE system, restriction enzymes, DNA sequencing after amplification of genomic DNA by PCR.
Results. Five β-thalassemia alleles (IVS-I-110 G-A, codon 39 C-T, IVS-I-6 T-C, IVS-I-1 G-A, codon 44 -C) were present in nearly 88% of the β-thalassemia alleles. The frequencies of these mutations were nearly similar to those found in other studies in Albanian population. 321 chromosomes with a β-thal mutation have been analyzed and 13 mutations were found in all studies carried out in Albanian population. Common point mutations as IVS-I-110, codon 39, IVS-I-6, IVS-I-1 and codon 44 were present in nearly 92% of β-thalassemia alleles. A genetic heterogeneity was detected from one study to another. A rare new mutation, IVS-I-2 was found for the first time in Albanian population in our study.
Conclusion. Five point mutations were present in nearly 92% of β-thalassemia alleles, which will facilitate a prenatal diagnostic program. It makes possible the detection of the heterozygotes for the β-thalassemia in Albanian population screening program and offers an accurate prenatal diagnosis with a probability 92%.

P0831. Correlation between the neural tube defects and mutations in the methyltetrahydrofolate reductase gene in the kazakh population

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Neural tube defects (NTD) are one the most frequent congenital anomalies (CA) in Kazakhstan. The frequency of such defects is 0.7 per 1000 newborns in Kazakh Republic. The dynamics of NTD frequency has not been displaying a declining tren from 1998 to 2005.
It is known that the methyltetrahydrofolate reductase (*MTHFR*) gene mutations play a substantial role in the NTD's etiology. In order to examine the causes of NTD's in the Kazakh population we have conducted a molecular-genetic study for carriage of *C677T* and *A1298C* mutations in the *MTHFR* gene. The parents of 84 newborn with NTD and 96 persons of ethnic Kazakh descent for the control group were studied.
In the main group, the favorable homozygote genotype (*CC)* occurred reliably less frequently - 53.5±5.4%, in comparison with the control group - 76,0 ± 4,4% (p<0,005). The frequencies of homozygote on the *T* allele (*TT*) (8,4 ± 3,0%) and heterozygote (*CT*) (38,1 ± 5,3%) in the main group has also reliably exceeded than the analogous index of the control group (3,2 ± 1,0 and 20,8 ± 4,1%;p< 0,05). Genotypic frequencies for *A1298C* polymorphism have not had differences between the main and the control group.
In the main group reliably low frequency of positive haplotype *C677/ A1298C* and high frequency of negative haplotypes *C677T/A1298C, C677T/ 1298C, 677T/ A1298 and 677T/A1298C* were observed.
The results state that there is a possibility of potential contribution of negative *MTHFR* gene types carriage to the etiology and risk of NTD development.

P0832. Epimutation at both the Beckwith-Wiedemann Syndrome and transient neonatal diabetes (TND) loci in two individuals ascertained from a cohort of TND patients

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Transient neonatal diabetes mellitus (TNDM) is characterised by intrauterine growth retardation, while Beckwith-Wiedemann syndrome (BWS) is a clinically heterogeneous overgrowth syndrome. Both TNDM and BWS may be caused by aberrant loss of methylation (LOM) at imprinted loci on chromosomes 6q24 and 11p15.5 respectively. We identified a cohort of individuals ascertained because of Transient neonatal diabetes, 17 due to paternal UPD 6, 9 with a duplication of chromosome 6, 13 with loss of maternal methylation at 6q24 (LOM) and 4 of unknown aetiology. We investigated the cohort for methylation aberrations at 11p15.5 and demonstrated that 2 patients, both with LOM at 6q24, had in addition loss of maternal methylation at KvDMR (domain 2) at 11p15.5. All other TNDM cases investigated showed normal methylation patterns at KvDMR, as did 120 controls. This shows that imprinting anomalies occasionally affect more than one imprinted locus.

P0833. Functional characterization of the first B-box missense mutation in TRIM37 underlying mulibrey nanism

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Mulibrey nanism is a rare congenital growth disorder with autosomal recessive inheritance. The characteristic features include, short stature, typical craniofacial features, progressive cardiopathy and failure of sexual maturation. The patients develop insulin resistance syndrome and type 2 diabetes and they have an increased risk of developing kidney, liver and ovarian tumors.
Mutations in *TRIM37*, a gene encoding for a novel RING finger ubiquitin E3 ligase, underlie mulibrey nanism. The family of TRIM proteins is characterized by the presence of tripartite motif, which includes a RING finger, one or two zinc-binding B-box motives and a coiled-coil region.
To date 12 *TRIM37* mutations have been characterized in mulibrey nanism patients of different ethnic origin. Most of these are truncating, with only two missense mutations (L76P, G322V) identified so far.
We studied three newly diagnosed patients and identified four novel mutations. Two patients are homozygous for two new truncating mutations, while the third patient is compound heterozygous for a B-box missense and a novel truncating mutation. The missense mutation is the first identified in the B-box domain of TRIM37. As it affects a cysteine residue and thus may affect the zinc-binding of the B-box,

functional characterization of the mutant protein may gain insight into the mechanisms underlying mulibrey nanism. Functional studies underway include subcellular localization, ubiquitin E3 ligase activity and interaction properties of the mutant protein.

P0834. Investigation of *TSC* genes mutation spectrum in tuberous sclerosis families in Taiwan

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Tuberous sclerosis (TS) is an autosomal dominant disorder characterized by the development of hamartomatous growth in many organs. In this study, twenty-seven Taiwanese families including 25 familial and 2 sporadic cases suffering from this disease were tested for mutations in the TSC1 and TSC2 genes. We use RT-PCR method to detect the presence of hamartin and tuberin mRNA from patients with TS and their parents. Mutation screening of the TSC1 and TSC2 genes of the TSC patients was performed with denaturing high-performance liquid chromatography (dHPLC), then DNA sequence variations were confirmed with direct sequencing. Data indicated that genetic lesions were found in 16 patients among the 27 patients. Three possible pathogenic mutations were found in the TSC1 gene, including one missense, one nonsense and one frame shift mutation. In the TSC2 gene analysis ten possible pathogenic mutations in the sporadic cases were found, including two in-frame deletion, seven deletions, one nonsense mutation, and two missense mutations. In addition, real-time quantitative PCR protocol was conducted to determine the quantities of hamartin and tuberin mRNA in those patients with mutations. Comparing patients with mutation and their parents, various levels of hamartin and tuberin mRNA were found. Besides, no significant correlation between particular clinical features and these mutations were found. In conclusion, it seemed that no mutation hot spot was present in Taiwanese TS patients. More data need to be collected to further support this finding.

P0835. *TBX3*, the Ulnar-mammary syndrome gene, plays role in mammary cell proliferation independently of *p19ARF* and *p53*

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TBX3, the gene mutated in the Ulnar Mammary Syndrome (UMS), is a transcription factor of the *T-box* gene family, known to inhibit transcription from the *p19ARF* promoter and contribute to cell immortalization. One of the main features of the UMS is the severe hypoplasia of the breast, associated to haploinsufficiency of TBX3. Based on the UMS phenotype it is logical to expect that *TBX3* may have a positive role in the proliferation of the mammary epithelial cells (MECs). In addition, in mice homozygous for the targeted disruption of *Tbx3* the mammary gland (MG) is nearly absent from early stages of embryogenesis, while in the heterozygous ones the MG show reduced ductal branching. These data strongly suggest a specific role of *TBX3* to promote growth of MECs, however, direct evidence in this direction is lacking. Here we show that *TBX3* has growth promoting function in several models of MECs, associated to its ability to repress the *p19ARF* promoter, while no direct effect of *TBX3* on cell differentiation or apoptosis could be observed. TBX3 represses transcription of *p19ARF* in MECs, however growth-promoting function of *TBX3* appears to be independent of the p19ARF, p53 and mdm2 cell-cycle regulatory proteins, as *p53*-null MEC show similar growth responses associated to up- or down-regulation of *Tbx3*. In fact the downregulation of *p19ARF* does not change proliferation, but rather reduces apoptosis of MECs. These are the first direct evidence that the level of *TBX3* expression positively controls proliferation of MEC using pathways alternative from *p19ARF* and *p53*.

P0836. Association Between IL4 (-590), ACE (I)/(D), CCR5 (Δ32) And IL-1RN (VNTR in Intron 2) Gene Polymorphisms And Vitiligo In Turkish Patients

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Vitiligo is a common skin disorder characterized by patchy depigmentation, due to the decrease of melanin pigment resulting from the apparent melanocyte loss. The pathogenesis is still unknown, but several hypotheses have been advocated. Recently polymorphisms in a number of genes which are involved in the immune system have been found to play a role in the susceptibility to vitiligo disease.

The aim of this study was to investigate interleukin 4 (IL4) gene (-590), angiotensin converting enzyme (ACE) gene insertion (I)/deletion (D), C-C chemokine receptor 5 (CCR5) gene (Δ32) and IL1-R antagonist (IL1-RN) (VNTR in intron 2) gene polymorphisms (GP) in Turkish vitiligo patients.

The study included 48 vitiligo patients and 50 healthy controls. Polymorphisms for the genes ACE, CCR5 and IL1-RN were detected by PCR. IL4 polymorphism was typed by PCR-RFLP. Genotypes and allelic frequencies for these genes in patient and control groups were compared.

No significant differences in either the genotype distribution or the allelic frequencies of IL4, CCR5 and ACE gene polymorphisms were observed between vitiligo patients and healthy controls. CTLA4 GG and IL1RA 2/2 genotypes (16.6%, 12.5%) and CTLA4 G allele frequency (26%) were found to be significantly higher in the vitiligo patients compared to the control group (p=0.044, 0.003, 0.000). CTLA4 AA and IL1RA 1/5 (64.6 %, 0%) genotypes and IL1RA 5 allele frequency (0%) were found to be significantly lower in the vitiligo patients compared to the control group (p=0.014, 0.013, 0.015).

P0837. Analysis of ATP7B gene exon 14 in Latvian Wilson disease patients

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Wilson disease (WD) is an autosomal recessive inherited disorder of hepatic copper metabolism resulting in liver disease and/or neuropsychiatric disease. WD is caused by mutations in the gene ATP7B. More than 200 mutations of this gene have been reported, the most common of them being H1069Q in the exon 14. This mutation is found in 30-80% of WD patients in different populations.

Our aim was to analyze exon 14 of ATP7B gene in Latvian WD patients. We studied DNA of 11 patients, whose symptoms and laboratory tests gathered 3 or more points by WD scoring system before mutation analysis. The liver copper quantification and rhodanine staining of hepatocytes is not available for our patients.

DNA was extracted from whole blood by standard phenol/chloroform purification protocol. The DNA analysis methods used were PCR, SSCP and direct sequencing.

Of 22 WD chromosomes analyzed 5 were found to carry the mutation H1069Q (two patients were H1069Q homozygotes, one heterozygote). Thus the frequency of this mutation in WD chromosomes in Latvia is 22.7%. The relatively low frequency of the mutation H1069Q may be due to the small patient group. In addition in one patient a previosly not reported mutation was found. The mutation 3106G>A involving first nucleotide of codon results in amino acid change V1036I. This patient did not carry mutations on the other allele of the exon. No other mutations were found in exon 14 of our patients. The causative role of mutation V1036I in development of WD still remains to be proved.

P0838. Detection His 1069Gln mutation in patients with Wilson disease using bidirectional PCR amplification of specific alleles (BI-PASA) test

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Wilson disease (WD) is an autosomal recessive disorder of hepatic copper metabolism caused by mutations in a gene encoding a copper-transporting P-type ATPase. The majority of known mutations affecting this gene are rare, except for the His 1069Gln mutation often found in patients of Northern or Eastern European origin. In our previous work we examined the frequency of the His 1069Gln mutation in Slovak

patients with Wilson disease using two different DNA-based methods: ACRS (amplification created restriction site) for Alw21I in combination with nested PCR, and ARMS (amplification refractory mutation system) test. Although, both mentioned methods are reproducible and precise, ACRS requires seminested PCR and digestion of PCR product and in ARMS test two PCR reaction must be performed to detect the His 1069Gln mutation. In the present work we describe a method based on bidirectional PCR amplification of specific alleles (Bi-PASA) which identifies His 1069Gln both in homozygotes and heterozygotes in one PCR reaction. Comparing results of BI-PASA test with ACRS and ARMS tests on 27 WD patients homozygous and heterozygous for His 1069Gln mutation and on 120 random DNA samples genotyped showed 100% concordance. In conclusion, BI-PASA is more simple and rapid method for detecting the of His 1069Gln mutation when compared to both ACRS and ARMS method.

P0839. Identification of X linked gene for Wiskott- Aldrich Syndrome (WAS) in irainain patients

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Wiskott-Aldrich Syndrome (WAS) is a rare inherited disorder that may be characterized by recurrent infections due to defects in the immune system (i.e., partial defects in T lymphocyte and B lymphocyte systems [combined immunodeficiency]); abnormal bleeding caused by a deficiency in circulating blood platelets (thrombocytopenia); a high incidence of “autoimmune-like” symptoms; and the presence of scaling, itchy skin rashes (eczema) that may be mild in some affected individuals and severe in others. The range and severity of symptoms and physical features associated with the disorder may vary greatly from case to case. Because Wiskott-Aldrich Syndrome is inherited as an X-linked(Xp 11.22-23) recessive genetic trait, the disorder is usually fully expressed in males only.

We want to investigate the frequency and variety of Xp 11.22-23 mutations that cause WAS in Iranian patient for the first time and to analyze Exon 1,2,4,7,10 and find the mutations so according to that we could design prenatal diagnosis kit.

P0840. A novel mutation of IL1RAPL1 in the Polish MRX family.

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Mental retardation (MR) affects approximately 2% of the population. It has been observed that affected males outnumber females of about 30%. This fact might be due to be an an effect of mutations of the genes located on X chromosome. Until recently mutations in about twenty genes are known to result in nonspecific form of X-linked mental retardation (MRX). Because MRX is a clinically homogenous but genetically heterogenous disorder, linkage studies in extended pedigrees followed by mutational analysis of known MRX genes in the linkage interval are very often the only way to identify a genetic background of the disorder.

We have performed linkage analysis in several MRX families and we have mapped one family to an interval encompassing Xp22.2-11.3 (LOD score >2). There are three known MRX genes located in this region: *ARX*, *IL1RAPL1* and *TM4SF2*. Following mutational analysis of those genes let us to identify a deletion of four exons (3, 4, 5, and 6) of the *IL1RAPL1* gene. In order to confirm the results of molecular analyses we performed cytogenetic studies. This enabled us to define the molecular background of the disease and to assess the carrier status of females. Here we present the results of molecular, cytogenetic, clinical and psychological analyses of four patients with deletion in the *IL1RAPL1* gene.

P0841. *ZNF674*: a novel X-linked mental retardation gene identified through array CGH

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Non-syndromic X-linked mental retardation (MRX) is a complex and highly heterogeneous genetic disorder. Some 25 MRX genes have been identified so far, which collectively explain about 20% of the patients in our cohort of families with MRX. For the identification of novel MR genes, array CGH has proven to be successful. We used full coverage X-chromosomal BAC arrays to reveal a 1 Mb deletion in Xp11.3 in a boy with a mental retardation syndrome. The deleted *SLC9A7*, *CHST7*, *ZNF673* and *ZNF674* genes were screened for mutations in 28 MRX families with a linkage interval that includes the Xp11.3 region. One nonsense mutation, p.E118X, was identified in *ZNF674* and therefore, another 306 unlinked XLMR patients were tested for mutations. Two more nucleotide changes, p.P412L and p.T343M, were identified. These three changes were not present in 350 control X-chromosomes. The p.E118X leads to a truncated protein that has no zinc finger domains. The Pro to Leu change is present in a conserved linker between two zinc finger domains and probably causes a disturbance of its DNA binding property. The second missense mutation p.T343M, involves an amino acid that is not conserved in other zinc finger genes. *ZNF674* belongs to a cluster of seven highly related zinc-finger genes in Xp11, two of which (*ZNF41* and *ZNF81*) have been implicated previously in MRX. Identification of *ZNF674* as the third MRX gene in this cluster may indicate a common role for these zinc-finger genes that is crucial to human cognitive functioning.

P0842. Influence of polymorphisms in xenobiotic-metabolizing and DNA-repair genes on diepoxybutane-induced SCE frequency

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Several studies on polymorphisms in genes exerting “minor impact” on susceptibility to xenobiotics indicated that analysis of combined polymorphisms might be the key to an understanding of the role of these genes in modulation of susceptibility to mutagens.

The aim of our study was to test for associations between DEB-induced SCE-frequency and genetic polymorphisms in genes coding xenobiotic metabolizing enzymes (*CYP1A1, CYP2, GSTT1,XPDE, NAT2)* as well as DNA repair proteins (*XRCC1, XRCC2, XRCC3, XPD, XPA, XPC, XPG, XPF, ERCC1, BRCA1, NBS1, RAD51*) in *in vitro* experiment on healthy donor’s lymphocytes. We found that *GSTT1* null genotype and c1/c2 variant of *CYP2E1* are associated with higher, while $G^{590}A$ variant of *NAT2* with lower SCE induction by DEB. No correlation between DEB-induced SCEs and any of tested variants in DNA-repair genes was revealed. The analysis on pairs of genes showed that for a fixed *GSTT1* genotype, the SCE level is increasing in the number of *tyr* alleles in *EPHX* position 113 (exon 3). It was also observed that among *GSTT1(+)* individuals the DEB-induced SCE level is significantly lower when the *EPHX,* codon 139 genotype is *his/arg* than *his/his*. An interaction between *CYP2E1* and *EPHX*, codon 139 polymorphisms was also observed. In *CYP2E1* c1/c2 individuals an increase in DEB-induced SCEs is lower when the *EPHX* is *tyr/his* than when it is *his/his.* In conclusion, our study supports the thesis that the analyses of networks rather than of a single gene are important in the understanding of complicated biological processes such as mutagenesis.

Po06. Genetic analysis, linkage, and association

P0843. Haplotype Block Analysis in 2q36 a Susceptibility Locus for Absense Epilepsy

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Childhood Absance Epilepsy (CAE) and Juvenile Absance Epilepsy (JAE) are subtypes of Idiopathic Generalized Epilepsy (IGE) that have a clear genetic component with an incomplete penetrance. Whole genome linkage studies resulted in succeptibility loci for CAE and JAE on chromosome 8q24, chromosome 5, 3q26, 14q23 and 2q36. Further studies in 3q26 resulted in the identification of a mutation in Cl channel gene in one family. A genome-wide scan in rat models linked 2q36 syntenic region to CAE. An other association study showed a low possibility of gene SLC4A3 in 2q36 to contribute to the etiology of CAE. Studies using candidate gene approach revealed a mutation in $GABA_A$ receptor, a voltage-gated Ca^{2+} channel gene in one family afflicted with CAE. Using haplotype block structure in an association study can be another approach to further clarify the involvement of 2q36 in absance epilepsy. Therefore, we aimed to identify haplotype block structure in the Turkish population using 33 SNPs in a 160 kb region using 35 trios. If haplotype blocks are identified, tag SNPs will be used to compare the patient samples with healthy controls. Herein, we present a preliminary haplotype structure with 6 blocks in the region analyzed with 21 SNPs in 18 trios. When all 33 SNPs are genotyped in 35 trios we expect to see a more definite block structure and the presence of recombination hot spots.

P0844. Genotype and phenotype correlation of diabetic patients and 2 years follow-up

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Diabetes is a chronic metabolic disorder with multifactorial etiology. Many Single nucleotide polymorphisms (SNP) are shown to participate in disease pathogenesis or progression. Among these, renin-angiotensin-aldosterone system (RAS) genes are involved in nephropathy development. The aim of this study is to investigate the RAS genotype and phenotype relation in T2DM patients and to study the effects of SNP's on progression to nephropathy.

In this study 60 patients' metabolic parameters (HbA1c levels) and renal functions (urinary albumin excretion, creatinine clearance, BUN and creatinine levels) were evaluated. The patients' ACE and AT1R gene polymorphisms were investigated. The patients were followed up by a period of 2 years and their metabolic parameters and renal functions were re-evaluated.

The patients' mean age was 56.85 ± 10.6 years. The mean HbA1c level, urinary albumin excretion and creatinine clearances were 7.72 ± 1.87%, 38.3 ± 71.9 mg/24h and 94.9 ± 23.4 mg/ml/1.73m^2, respectively. BUN and creatinine levels were in normal limits in all of the patients. No significant relation was found between genotypes and these baseline metabolic and renal function parameters. Fourty four patients were followed for 20.84 ± 6.9 months. The alteration in the parameters was not statistically significant (paired t test $p>0.05$). In 5 patients urinary albumin excretion were deteriorated. One patient was DD homozygous and the other 4 was ID heterozygous. HbA1c levels were also worsened in all of these patients. Therefore, in order to investigate the relationship between genotypes and progression to nephropathy, follow-up for a longer period is necessary.

P0845. Exclusion of *CNGB3*, *CNGA3* and *GNAT2* genes of complete achromatopsia in a consanguineous Tunisian family

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Complete achromatopsia also referred to as rod monochromacy is a rare (1/30000 to 1/50000) congenital stationnary retinal disorder characterized by a complete inability to discriminate between colours, a photophobia, a nystagmus and reduced visual acuity.

Achromatopsia is genetically heterogeneous with variable expressivity. The complete form is recessively inherited. The three achromatopsia genes are *CNGA3*, *CNGB3* which respectively encode for α and β subunits of the cGMP gated cation channel in cone cells and *GNAT2* which encodes the α subunit of cone transducin.

The transducin and the cGMP gated cation channel are important for the amplification and the transmission of visual signal from cones to the cortical regions.

We report here genetic analysis of a large consanguineous Tunisian family with nine individuals presenting with complete achromatopsia. Genetic linkage to the three genes accounting for the majority of achromatopsia *id est CNGB3 CNGA3* and *GNAT2* was investigated. For this purpose microsatellite markers overlapping these genes loci were selected D8S273, D8S167 and D2S2311, D2S2175 and D1S2849, D1S412 for *CNGB3*, *CNGA3* and *GNAT2* genes respectively.

Genotyping and linkage analysis excluded linkage to these genes. These results are confirmed by a statistical analysis of Lod Score.

A genome wide scan is currently being performed to allow identification of a potential new locus for complete achromatopsia.

P0846. Analysis of conserved non-coding sequences (CNSs) led to the finding of a 60Mb long inversion on the X-chromosome in patients with Addison's disease and hypogonadotropic hypogonadism

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The co-occurrence of Addison's disease and hypogonadotropic hypogonadism has been related to intragenic mutations in the *DAX1*-gene at Xp21.2 (Dosage sensitive sex reversal, adrenal hypoplasia congenita, critical region on the X chromosome, gene 1). This gene encodes a transcription factor, which together with SF1 (Steroidogenic factor 1), is essential for normal development of the adrenal cortex and gonads.

The aim of this study was to map the mutation that led to an X-linked form of Addison's disease and hypogonadotropic hypogonadism (HH) in a family with four affected males. The patients had apparently normal karyotype and no mutations in the *DAX1*-gene (exons, intron, promoter). X-chromosome linkage analysis was followed by analysis of conserved non-coding sequences (CNSs) upstream of the *DAX1*-gene promoter. This led to the discovery of a 60Mb long pericentric inversion, where one of the breakpoints for the inversion was located 3.8kb upstream of *DAX1* transcription start. To our knowledge this is the first report of an inversion on the X-chromosome that causes Addison's disease and HH. The active use of CNSs to identify disease-causing mutations in non-coding DNA sequences has to our knowledge only been reported successfully once in a study by Lettice *et al*. (Hum Mol Genet., 2003).

The detected inversion leads to dislocation of a previously predicted conserved SF1 consensus transcription binding site. Immunohistochemisty showed a seemingly normal DAX1 expression, but a complete absence of SF1, in testis tissues from the patients with the inversion. Further work is ongoing to determine the mechanisms behind these observations.

P0847. Mutational and linkage analysis in three Calabrian families with Autosomal Dominant Nocturnal Frontal Lobe Epilepsy

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Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is caused by mutations in the genes encoding the α4 and β2 subunits of the neuronal nicotinic acetylcholine receptor (*CHRNA4* and *CHRNB2*, respectively). However, only a minority of ADNLE families carry a

mutation of either gene. A third ADNFLE locus has been identified but mutations linked to the disease have not yet been found. Very recently, variations in the promoter of the corticotropic-releasing hormone gene (CRH) have been reported in sporadic and familial patients. Here, we investigated whether nine brain-expressed genes, encoding distinct neuronal nicotinic acetylcholine receptor (nAChR) subunits, and CRH are associated with the disease in three ADNFLE families from Southern Italy.
Firstly, exon 5 of CHRNA4 and CHRNB2 genes, harboring all the known mutations, were sequenced in the probands and found negative. Then, we performed a linkage study on 11 affected and 29 non affected individuals belonging to these three families with microsatellite markers and RFLPs encompassing the chromosome localization of the nAChR subunit genes and CRH. The following chromosome regions were examined: 1q21 (CHRNB2), 8p21 (CHRNA2), 8p11.2 (CHRNA6, CHRNB3), 15q14 (CHRNA7), 15q24 (CHRNA5/A3/B4), 20q13.2 (CHRNA4), 8q13 (CRH). Two point and multipoint linkage analyses, performed by LINKAGE and GENEHUNTER, allowed us to exclude the association between ADNFLE and all the examined genes, except in one family in which a mutation screening in CRH is still in progress. These results further illustrate the considerable genetic heterogeneity for such a syndrome, despite the quite homogeneous clinical picture.

P0848. The influence of VEGF polymorphism on the progression of ADPKD

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A significant phenotypical variability is observed in autosomal dominant polycystic kidney disease (ADPKD), the most common hereditary kidney disease. Vascular endothelial growth factor (VEGF) is a potent angiogenic agent, a mitogen for endothelial cells. Dysregulation of VEGF expression in the kidney has been demonstrated in a wide range of primary and acquired renal diseases. Higher VEGF expression was found in polycystic kidneys.
We examined the influence of the 2578 C/A and the 1154G/A polymorphism in the regulatory region of the VEGF gene on the progression of ADPKD towards end stage renal disease (ESRD). The -2578C and -1154G allele were described to be associated with higher VEGF production. 287 ADPKD patients who had reached ESRD were analyzed. Patients were divided into three groups: 1. with ESRD later than in 63 years , 2. with ESRD before 45 years and 3. with ESRD between 45-63 years. We compared the frequencies of different genotypes between the groups.
The VEGF 2578 C/A genotype distribution showed no differences among the groups. We observed nonsignificantly higher tendency to later ESRD in AA females (55.1±8.2) in comparison with CC females (51.7±9.4) (t-test, p=0.09).
The VEGF 1154 G/A genotype distribution showed no differences among the groups. We observed nonsignificantly higher tendency to later ESRD in AA females (56.3±9.1) in comparison with AG females (51.9±6.9) (t-test, p=0.07).
To conclude, we excluded significant influence of 2578 C/A and 1154 G/A VEGF polymorphisms on the progression of ADPKD.
IGA MZ CR 7633, 7733 and ZZ MSMT 0021620806.

P0849. Association of a dopamine transporter gene (DAT1) polymorphism with alcohol dependence in Russian population of West Siberia

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VNTR-polymorphism in 3' UTR of the dopamine transporter gene was examined in patients with alcoholism combined with tuberculosis (n=84) in comparison to control population sample (n=122). The genotype frequencies obeyed the Hardy-Weinberg equilibrium in both groups. The *DAT1* VNTR-polymorphism was significantly associated with an increased risk of comorbidity of alcoholism and tuberculosis in Tomsk population (maximum-likelihood χ^2=10.75, *P*=0,030). The frequencies of the allele with 8, 9, 10 and 11 repeats in patients were 1.2%, 16.1%, 82.1% and 0.6% respectively. Allele of 8, 11 repeats weren't revealed in control group. The frequency of the 9, 10 allele was 24.2%, 75.8% in this group respectively. Odds ratio (*OR*) and 95% confidence intervals (*CI*) in individuals with 9 repeats allele (genotypes: 8/9, 9/9, 9/10; n=25 and n=54 for disease and control groups accordingly) in comparison with other individuals (10/10 + 10/11 groups; n=59 and n=68 in patients and controls accordingly) for significantly increased risk of comorbidity of alcoholism and tuberculosis is 0.40 (95% *CI* 0.21 - 0.75; *P*=0.002). It is possible that the 3' untranslated VNTR functions in the control of expression of the *DAT1* gene.

P0850. Frequency of α-thalassemia mutations among Iranian populations

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Alpha thalassemia is one of the most common hemoglobin gene disorders which is due to deletions or point mutations of α-globin gene. Present study was designed to determine the spectrum of common α-thalassemia mutations in Iran.
In present study, total number of 406 patients referred to our center from 1998 till 2005 with hypochromic microcytic anemia and normal HbA2 level who were shown to be negative for β-thalassemia genotyping were choosen. They were tested for nineteen most common α-thalassemia mutations. Analysis for common deletions was performed using gap-PCR amplification followed by agarose gel electrophoresis of the resulting PCR fragments. Additional mutations were analyzed by reverse dot-blot and by DNA sequencing of alpha 1 and alpha 2 gene.
Due to this comprehensive study, it could be stated that -α3.7 mutation is the most frequent α-thalassemia mutation in our population and contributed about 62% of α-thalassemia mutations in Iran. However, an additional 8 mutations (--Med, $-\alpha^{4.2}$, $\alpha^{Pa1(GAA)}$, $\alpha\alpha^{cs}$, α^{-5nt}, $-(\alpha)^{20.5}$, $\alpha^{Pa2(AAG)}$, $\alpha\alpha^{cd19}$) significantly cover 8.3% to 1.3% of mutations and the remaining 5 mutations consider to be very rare. Northeast has the highest rate of -α4.2 mutations, on the contrary to southeast and west which no 4.2 mutation have investigated. Also it could be stated that in (17.7%) of individuals no mutation figured out, which could be β-thalassemia silent or carriers of other type of α-thalassemia. Since 2004 by use of Alpha-globin Strip Assay we could improve the identification of the alpha mutations significantly (76% before 2004 , 88% in 2004).

P0851. Interaction Between the Cholesteryl Ester Transfer Protein (CETP) Gene and APOE in Late Onset Alzheimer's Disease.

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Late onset Alzheimer's disease (LOAD) is a complex neurodegenerative disorder. Of the many genes studied thus far with respect to the disease, the only consistently replicated genetic risk factor has been the APOE gene. The APOE*4 allele of this gene may promote amyloid beta (Aβ) plaque fibrillation, which is one of the hallmarks of LOAD. APOE may relate to LOAD through cholesterol by regulating the production of Aβ, with high cellular cholesterol reducing Aβ levels. Cholesteryl ester transfer protein (CETP) is involved in the same pathway through regulation of the HDL levels. We studied both the APOE gene and the I405V polymorphism of the CETP gene in relation to LOAD and investigated whether this polymorphism is independently associated with LOAD risk, or acts in concert with the APOE gene. We genotyped 525 LOAD cases and 5404 controls from the Rotterdam Study using a TaqMan allelic discrimination assay. Our results show that in the APOE*4 non-carriers group, those homozygous for the V allele of the I405V polymorphism have a 1.6 fold (95% CI 1.1-2.5, p = 0.02) increase in risk of LOAD compared to non-carriers. This risk

increases to 2.5 (95% CI 1.4-4.5, p = 0.001) in the APOE*4 carriers group (p value = 0.04 for gene-gene interaction). Our results suggest that the interaction of CETP with APOE increases the risk of LOAD, probably through cholesterol metabolism in the brain.

P0852. Androgen receptor gene $(CAG)_n$ polymorphism in patients with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a multifactorial oligogenic trait, with chronic anovulation and clinical and/or biochemical hyperandrogenism as main characteristics. The gene encoding androgen receptor (*AR* gene) (Xq11-q12) has been proposed as being a candidate gene for susceptibility to the syndrome. The $(CAG)_n$ repeat polymorphism in exon 1 of *AR* gene was found to be inversely correlated to the gene's transcriptional activity and consequently to the transactivation potential of androgen receptor. The aim of the present case-control study was to evaluate the incidence of the polymorphism in Slovene PCOS patients. One hundred seventeen PCOS patients and 110 age-matched fertile controls were genotyped for the polymorphism, using polymerase chain reaction. Serum total testosterone (TT) levels were determined by radioimmunoassay, and body mass index (BMI) was calculated. Considering either both of the two *AR* alleles or biallelic means, the distributions between PCOS and control patients were of no statistical significance ($P = 0.334$ and $P = 0.376$, respectively). In PCOS women, BMI and serum TT levels were significantly positively correlated ($R = 0.276$, $P = 0.002$). However, the number of CAG repeats could not explain the variability in serum TT levels in PCOS patients with statistical significance (after adjustment for BMI, $P = 0.938$, adjusted $R^2 = 0.07$). To conclude, the $(CAG)_n$ *AR* repeat polymorphism is probably not a key risk factor for the development of PCOS in Slovene patients, but its indirect involvement in the pathogenesis of PCOS, by complex interactions with other susceptibility factors might not be excluded.

P0853. Association of the ACE genetic polymorphism and the maximal oxygen consumption of human

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Angiotensin converting enzyme (ACE) is a key component of the circulating human reninangiotensin system (RAS) and also adjusting activity of cardiovascular system. In intron of the 16th gene's was founded the polymorphism (17q23), caused insertion (I) - 287 p.n. or deletion (D) of the Alu-element (Rigat et.all., 1992).

There are three polymorphic genotypes - I/I, I/D, D/D. The purpose of research was studying the association of the given polymorphism with a level of the physical working capacity; one of the parameters is the maximal consumption of oxygen (MCO).

Among the investigated 70 persons by men with high parameters MCO is observed higher frequency of occurrence allele I (46.2%, 10.1%, P=0.014) and decrease in frequency of occurrence of a heterozygotic genotype in comparison with the control. In a group with low values MCO authentic increase of frequency of occurrence of homozygous genotype D/D is established (87.8%, 14.2%, P=0.014).

Thus influence of polymorphism in gene ACE on level MCO is arranged.

P0854. Ant-egg-cataract - distinct phenotype for an autosomal dominant congenital cataract first described in a Danish family in 1967

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A Danish family described in 1967 (Riise; Acta Ophthalmol 1967:45:341-6) presents a distinct phenotype of congenital cataract in which globular structures resembling ant-eggs are found in the lens. EM studies of these ant-egg-like inclusion bodies (Schrøder; Nissen; Acta Ophthalmol 1979:57:14-9 and 57:435-42) showed a central core consisting of calcium-crystal-like structures surrounded by a transition zone containing membrane-limited cytoplasmic bodies and linear five-layered structures. Genome wide linkage analysis was applied to identify the disease causing mutation.

Materials: Blood was collected from 21 members of the family and DNA isolated.

Methods: A complete genome-wide linkage scan was carried out using the ABI md-10 Linkage Mapping set and ABI3100 sequencer technology. DNA sequencing was performed using ABI BigDye and an ABI377, and confirmation of the mutation in family members was carried out using restriction enzyme digests.

Results: A LOD score of 3.91 was obtained for D13S1275 close to the gap junction gene *GJA3* (*CX46*), and sequencing of the DNA from two affected members of the family discovered the mutation L11S, c.32T>C, in exon2. The L11S amino acid change is located in the protein signal peptide, and is the only *CX46* mutation known to that affect the signal peptide. The connexins are integral membrane proteins involved in contact and transport between neighbouring cells. The mutation in the *CX46* signal peptide seems likely to explain the formation of the inclusion bodies found in the lenses if the altered signal peptide disturbs the intracellular trafficking of the premature protein through the Endoplasmic Reticulum and Golgi Apparatus.

P0855. Apolipoprotein A5 gene promoter T-1131C polymorphism associates with elevated triglyceride levels and confers susceptibility for ischaemic stroke

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The possible role of triglycerides (TG) in the pathogenesis of ischaemic stroke is still under extensive investigation. Recently, apolipoprotein A 5 (apoA5) gene promoter region T-1131C polymorphism has been shown to be associated with elevated serum triglyceride levels. In our work a total of 302 subjects were classified as large vessel associated, small vessel associated or mixed group of ischaemic stroke patients. As a control group, 289 stroke and neuroimaging alteration-free Caucasian subjects were examined. The level of TG was increased in all patient groups versus the controls ($p<0.05$). The apoA5 -1131C allele frequency was approximately two-fold in all groups of stroke patients compared with the controls (5 vs. 10-12%; $p<0.05$); and the apoA5 -1131C allele itself was also found to be associated with increased TG levels in all groups. In multivariate logistic regression analysis model adjusted for differences in age, gender, serum cholesterol, hypertension, presence of diabetes mellitus, smoking, drinking habits and ischaemic heart disease, a significantly increased risk of developing stroke disease was found in patients carrying the apoA5 -1131C allele ($P<0.001$; OR= 2.5 (1.3-4.7)); this association was also proven for all subtypes of the stroke. The results presented here suggest that the apoA5 -1131C allele is an independent risk factor for the development of stroke. Being the apoA5 is under the control of the peroxisome proliferator-activated receptor-α the current observations theoretically can have long term therapeutical consequences.

P0856. Apolipoprotein E polymorphism and colorectal cancer

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Apolipoprotein E (ApoE) gene polymorphism plays a major role in lipid metabolism. It has been suggested that ApoE polymorphism might regulate colorectal tumor risk and development. We examined this notion for colorectal cancer (CRC). ApoE genotype was determined in 223 patients with CRC and 300 healthy controls from Kocaeli region of Turkey using a PCR-RFLP method . When alleles and genotypes of 223 patients with CRC were compared with those of

300 healthy controls. No association was obtained in patients with CRC (OR=1.011; 95%CI=0.713-1.434; P=0.94). The distribution of the ApoE2/2, E2/3, E2/4, E3/3, E3/4 and E4/4 genotypes was 0.3%, 12.0%, 5.3%, 65%,16.7% and 0.7% in controls and 0.0%, 13,5%, 1.8%, 68.6%, 15.2%, and 0.9% in patients with CRC. The frequency of the ε2, ε3, and ε4 alleles was 9%, 79.34% and 11.67% in the controls and 7.62%, 82.96% and 9.41% in patients with CRC.In conclusion, ApoE polymorphism is not a genetic risk factor for CRC in Turkey.

P0857. Investigation of seven Known loci associated with non-syndromic autosomal recessive mental retardation and microcephaly based on STRs Marker Homozygosity mapping in 30 families

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Microcephaly is defined as a reduction in head circumference and this clinical finding infers that an individual has a significant diminution in brain volume. Microcephaly can be usefully divided into primary microcephaly, in which the brain fails to grow to the correct size during pregnancy, and secondary microcephaly, in which the brain has the expected size at birth but subsequently fails to grow normally. Hereditary microcephaly can be classified as X-Linked or autosomal, and syndromic or non-syndromic. Seven out of ten Non-Syndromic Autosomal Recessive Mental Retardation (NS-ARMR) loci are associated with microcephaly, including MCPH1-MCPH6 which belong to the family of MCPH (autosomal recessive primary microcephaly), and ARFGEF2.

The objective of this study was to investigate seven known loci associated with autosomal recessive mental retardation with microcephaly in 30 Iranian families. These 30 families have been selected from 264 families which referred from different parts of Iran to the Genetics Research Center. Each family was subjected to complete clinical examinations and karyotype abnormalities have also been excluded.

We performed Homozygosity mapping by using STRs (Short Tandem Repeats) markers. So far we analyzed 17 families for MCPH5 locus, out of which, one family was linked to this locus.

P0858. Linkage analysis for 50 Iranian families with autosomal recessive non-syndromic hearing loss for DFNB21 locus

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Background: Congenital hearing loss occurs in 1 out of 1000 to 2000 births and about 50% of all cases are estimated to be of genetic origin. About 70% of hereditary hearing loss is non-syndromic with autosomal recessive inheritance accounting for 80% of the genetic load. To assess the importance of responsible loci in Iranian population, we screened 50 consanguineous families with Autosomal Recessive Non-Syndromic Hearing Loss (ARNSHL)for DFNB21, which was linked to ARNSHL in Middle East countries.

Materials and Methods: 50 consanguineous families with at least three affected children, previously excluded by mutational analysis from GJB2 and GJB6 genes, were included in this study. We used three polymorphic markers including D11S1998, D11S4464, and D11S1299 in this study.

Results: Three families were linked to DFNB21 and two novel mutations have been detected so far. In two families a 266 Del T mutation and a large 9611bp deletion that starts from intron 9 and includes exon 10 in TECTA gene were detected.

Conclusion: This study showed that mutations in DFNB21 locus are the third common cause of ARNSHL in Iranian population. It seems that DFNB21 may play an important role in genetic load of ARNSHL in Iran. This will be confirmed by screening more families for this locus in Iranian deaf population.

P0859. Plakophilin-2 mutations are the major determinant of familial arrhytmogenic right ventricular cardiomyopathy in the Netherlands

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In at least 50% of cases Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a familial disease with an autosomal dominant mode of inheritance. Diagnosis is based on clinical criteria proposed by a Task Force in 1994. Recent identification of mutations in the plakophilin-2 (PKP2) gene in 27% of ARVC patients suggests an important role for this gene in this disorder. This will facilitate early identification of persons at risk. Our goal was to assess the prevalence of PKP2 mutations in patients with (supposedly) ARVC in four Dutch tertiary referral centers.

A total of 96 index patients were analyzed; 56 patients fulfilled ARVC criteria whereas 40 did not. The PKP2 gene was screened for mutations using DGGE/DHPLC and direct sequencing. In 24 out of 56 index patients (43%) fulfilling the criteria, mutations were identified. Four different mutations were found more than once: 2386T>C, 235C>T, 397C>T and 2489+1G>A. Haplotype analyses revealed that all of these were founder mutations. Of the 14 different mutations identified, 4 were missense, 7 nonsense, 2 insertion-deletion and 1 splice-site mutation. Eleven of these were novel. Mutations were found in 17/23 (73%) proven familial ARVC patients, whereas 11 proven non-familial cases, were non-carriers. In 2 out of 40 patients (5%) not fulfilling ARVC criteria, mutations were identified.

In conclusion, PKP2 mutations can be identified in up to 43% of Dutch patients fulfilling Task Force criteria for ARVC. Identical mutations occur frequently. These results will facilitate early recognition of persons at risk and future studies of potential genotype-phenotype relationships.

P0860. Screening ARX Gene in Iranian Families with X-linked Mental Retardation

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The recently identified gene *ARX* (*Aristalles-Related Homeobox*), codes the ARX protein, an important transcript factor that belongs to one of the three largest classes of homeoproteins, the paired (*Prd*) class. Several mutations have been identified in *ARX* gene, which are responsible for a wide spectrum of phenotypes, including both nonsyndromic X-linked mental retardation (MRX), and syndromic (MRXS) forms such as X-linked lissencephaly with abnormal genitalia (XLAG), Partington syndrome and X-linked infantile spasm syndrome. The most common mutation in *ARX* gene identified is a duplication 24 bp (24 bp dup) in exon 2. This duplication leads to an expansion of the second polyalanine tract of ARX protein.

The aim of this study is to obtain the relative prevalence of *ARX* mutations in the families which fragile X has been ruled out with established or putative X-linked mental retardation (XLMR) pattern.

Up to now, we have collected 60 probands from 60 families with two or more affected individuals obtaining informed consent form has been from the parents of probands. The chromosomal analysis by standard cytogentics method, did not show any abnormality. As the first step these families were screened for the most common form of

mutation, 24 bp dup, and follow by SSCP(Single Strand Conformation Polymorphisms) and DNA sequencing for the entire ARX gene. So far we have identified one individual with 24 bp dup.

P0861. A fast and powerful method for pedigree-based quantitative trait loci association analysis

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Studies of quantitative traits (QTs) using pedigrees are a promising approach to identification of novel loci (QTLs) related to human health and disease. Pedigree-based QTL association analysis using information on allele transmission (TDT-like approach) is fast, reliable and extendable, but when ethnic stratification can be ignored, the measured genotype approach may have greater power. In the latter approach, a marker studied for association is tested as a covariate in a polygenic model accounting for additive genetic relationships within the pedigree. However, in complex genealogies (which are especially typical for genetically isolated populations) the measured genotype analysis may be very time consuming, which makes genome screening impractical. We have developed a new and fast step-wise method for genome-wide pedigree-based QTL association analysis. The idea of the proposed method is to perform polygenic analysis using complete pedigree information and to use residuals as a novel QT, which can be analysed using conventional regression analysis. Using simulated and real data we show that the non-centrality parameter, which links directly to the power, may be two to four times greater for measured genotypes compared to the TDT approach. When no QTL is present, the step-wise approach is slightly conservative, but when QTL is present it's statistical power is close to that of the measured genotypes approach. Our method provides a fast, powerful and relatively simpe means of undertaking a genome-wide QTL association analysis in general pedigrees that facilitates empirical significance testing strategies and may also be used for 2D genome screening for interacting QTL.

P0862. Phenome and syntropic genes

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Like 100 years ago, exploration of relationships between phenetic and genetic variability, genome and phenome, is still of current importance. Sequencing of human genome has shown that an important practical difference between genome and phenome is that while the genome is bounded (approximately 3 bilion base pairs), the phenome is not (its bound depends on how far we wish to go) (Raigen, Eppig, 2000). In thirties of last century, famous Russian geneticist Serebrovsky foreknew this while formulating statement about "unity of infinite number of traits and finite number of genes". In postgenomic era, phenotyping takes on special significance in resolving of the problem. Our approach to phenotyping for common diseases gene mapping is based on the phenomenon of polypathies.

Phenomenon of combination of several diseases in one individual and/or in its relatives was noted in XIX century and was reflected in the idea of "arthritism" (Bouchard, 1890). Later in the beginning of XX century this idea was developed by German pathologists who proposed to designate combined diseases by the term "syntropy" (Pfaundler, 1921). Such "combinations" are perhaps non-random and have evolutionary genetic basis. We call genes which are involved in susceptibility to such disease combinations as genes of syntropies (Puzyrev, 2000). Thus, combined diseases (syntropies) comprise those units of phenome that might correspond to some gene set, sample of co-regulated genes with similar structure (syntropic genes).

We apply this approach to analysis of relationships between genome and phenome, studying such syntropies as cardiovascular continuum diseases and metabolic syndrome, autoimmune syntropy.

P0863. Association study of the candidate region 9p22 in Italian families with asthma

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In a previous genome scan for asthma in 123 Italian families, phenotyped for skin prick test positivity to common aeroallergens (SPT), total serum elevated IgE (IgE), bronchial hyperresponsiveness to methacholine (BHR), clinical asthma and rhinitis, linkage on chromosome 9p22 has been detected for SPT or for IgE (Am J Hum Genet. 2002, Vol. 71 n. 4 Abs 1662).

We now performed a fine mapping and association study in this region using a set of 19 families that presented non-negative linkage for SPT or IgE.

Twenty-five SNPs spanning a region of 2.6 Mb harbouring several candidate genes were seleceted and analyzed.

Patients were genotyped by Minisequencing (11 SNPs), melting curve analysis of fluorescent real time PCR (4 SNPs) or enzimatic restriction (10 SNPs).

An association study was performed by the Trasmision Disequilibrium Test (TDT).

Data show TDT significant associations after permutation test, among which rs2584538 (p= 0.0079 for asthma), rs1332712 (p= 0.008 for IgE), rs1889088 (p= 0.00079 for BHR; p= 0.0066 for IgE, p= 0.0023 for rhinitis; p= 0.0094 for SPT), rs960232 (p=0.001 for rhinitis).

We will perform haplotype analysis and will extend the genotyping of the relevant polymorphisms on a larger sample of families.

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P0864. The *ATGL* gene is associated with free fatty acids, triglycerides and type 2 diabetes

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Adipose triglyceride lipase (*ATGL*) was recently described to predominantly perform the initial step in triglyceride hydrolysis and therefore seems to play a pivotal role in the lipolytic catabolism of stored fat in adipose tissue. In the first study investigating genetic variations within the *ATGL* gene in humans, twelve polymorphisms identified via sequencing and database search were studied in 2434 individuals of European ancestry from Utah. These polymorphisms and their haplotypes were analyzed in subjects not taking diabetic medication for association with plasma free fatty acids (FFA) as primary analysis, as well as triglycerides and glucose as a secondary analysis (n=1701, 2193 or 2190 respectively). Furthermore, type 2 diabetes (T2DM, n=342 out of 2434) was analyzed as an outcome. FFA concentrations were significantly associated with several SNPs of *ATGL* (p-values from 0.015 to 0.00003), consistent with additive inheritance. The pattern was similar when considering triglyceride concentrations. Furthermore, two SNPs showed associations with glucose levels ($p<0.00001$) and risk of T2DM ($p<0.05$). Haplotype analysis supported and extended the shown SNP association analyses.

These results complement previous findings of functional studies in mammals and elucidate a potential role of *ATGL* in pathways involved in components of the metabolic syndrome.

P0865. The dyslipidemia and obesity associated gene, FOXC2, contributes to atherosclerotic changes at the vessel-wall level

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Transcription factor gene forkhead box C2 (*FOXC2*), a key regulator of adipocyte metabolism, has been associated with coronary heart disease related risk factors such as variation in plasma lipid levels and obesity. To extend the assessment of the significance of *FOXC2*

gene from lipid levels of serum to quantitative atherosclerosis at the vessel-wall level, we studied informative variants across the *FOXC2* gene locus in an autopsy study sample with extensive measurements of arterial atherosclerotic changes of the coronaries, abdominal aorta and vessels of brain.
The autopsy study sample consisted of 700 males 33 to 70 years old with sudden death in Helsinki area. In the analysis of covariance, the *FOXC2* gene showed association with coronary narrowings (P=0.02), calcified (P=0.0007) and complicated (P=0.02) lesions of the coronary arteries and atherosclerosis of the brain (P=0.01). The data suggest that the association of *FOXC2* gene becomes evident especially in the more severe and progressed states of atherosclerosis. Furthermore, the strongest association was observed with the *C-512T* -polymorphism, which has shown suggestive functional importance in a previous study. Our results underline the importance of the *FOXC2* gene as a risk factor for dyslipidemia and atherosclerosis and give more insight into the possible pathogenesis associated with these genetic variants. In our autopsy data, a gene that earlier associated with plasma lipid levels subsequently associated with severe atherosclerosis at the vessel-wall level. Further functional studies are needed to truly establish the role of different *FOXC2* variants in lipid metabolism related diseases.

P0866. Association of SNP in the TNFSF4 gene with coronary artery disease and myocardial infarction in Italian population

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Atherosclerosis, the leading cause of death in developed countries, is the pathological basis for coronary artery disease (CAD), acute myocardial infarction (AMI), cerebral infarction, and peripheral vascular disease. Recent functional studies in a murine model, suggested a possible role of TNFSF4 (tumor necrosis factor ligand superfamily member 4), also called OX40 ligand (OX40L), in atherogenesis. The OX40L generates costimulatory signals by interacting with OX40 on T lymphocytes and it enhances the proliferation and differentiation of T lymphocytes, important processes in the initiation, progression and destabilization of atherosclerotic plaque. The human TNFSF4 gene, encoding the OX40L, was mapped to 1q24.3-25.1. Recently, in two independent Swedish population was showed the association of rs 3850641 A>G SNP, located in intron 1 of the TNFSF4 gene, with AMI susceptibility. To confirm this association in Italian population, we screened a group of 150 individuals with AMI, 150 individuals with CAD angiographically documented and 100 controls with angiographic evidence of clean coronary arteries. All subjects were genotyped by allelic discrimination assay (Taqman assay). Our data confirm the association of the rs 3850641 A>G SNP with susceptibility to AMI. In particular the G allele was significantly more represented in AMI individuals than in controls (P=0,003). Furthermore the allelic frequency distribution found in patients with AMI was similar to total group of patients (CAD + AMI). The present study confirms the association of this SNP with AMI susceptibility and extends the association to CAD susceptibility, providing evidence that TNFSF4 gene product is involved in the process of atherogenesis.

P0867. Haplotypes in ataxia-telangiectasia Italian patients reveal ancestral founder effects

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Ataxia-Telangiectasia (AT) is a rare autosomal recessive disease caused by mutations in ATM gene on chromosome 11q23.1. The ATM gene is very
large, spanning a genomic region of 150 kb, and is comprised of 66 exons. Analysis of short tandem repeat (STR) haplotypes of Italian ataxia-telangiectasia (AT; MIM# 208900) patients was carried out in 25 unrelated
families carrying six common AT mutations, 8283delTC, 7517delGAGA, 3894insT, 3802delG, 3576G>A (K1192K), and 8977C>T (R2993X). Haplotypes were characterised using five microsatellite markers, either intragenic or flanking the ATM gene (D11S1819, NS22, D11S2179, D11S1778 and D11S1294). This analysis has shown that patients carrying the same mutation also shared the same STR haplotypes, in agreement with a founder effect. Taken together, these mutations accounted for more than 35% of the Italian AT patients. These observations indicate that STR haplotypes prescreening should be part of ATM genetic testing protocol in the Italian population.

P0868. Association of CARD15 Polymorphisms with Atopy-Related Traits in a Population-Based Cohort of Caucasian

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Background: Influences of microbial pathogens are crucial for the maturation of the immune system. CARD15 is a cytosolic receptor involved in bacterial recognition by APCs. CARD15 polymorphisms have been associated with Crohn's disease. Recently, associations with atopic phenotypes have been reported in children.
Objective: Within a large population of German adults (n=1875) we evaluated eight CARD15 polymorphisms for associations with atopic phenotypes.
Methods: Subjects were phenotyped by standardized questionnaires and interviews as well as total and allergen-specific IgE measurements. Genotyping was performed using MALDI-TOF MS (Matrix-Assisted Laser Desorption Ionization - Time of Flight mass spectrometry). Haplotypes were estimated using the SAS/Genetics module.
Results: Subjects with a T allel at rs1077861 had a decreased risk to develop asthma (OR=0.648, p=0.013), whereas presence of an A allele at rs3135500 was significantly associated with an increased risk (OR=1.343, p=0.036). In addition, a CARD15 haplotype revealed to be protective against the development of asthma (OR=0.326, p=0.003). Subjects with an A allele at position rs5743266 or a T allele at rs2066842 had a significantly decreased risk to develop allergic rhinoconjunctivitis with ORs of 0.820 (p=0.049) and 0.801 (p=0.025). Polymorphism rs2066845 showed a significant association with increased total serum IgE (OR=2.155, p = 0.006).
Conclusion: Genetic variants of CARD15 that might result in inappropriate immunomodulation are not only associated with autoimmune diseases but also with atopic disorders.

P0869. Locus heterogeneity in ASD associated with AV-block

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The prevalence of congenital heart defects is approximately 1% of all live births. Identifying the genes responsible for cardiac malformation is the first step to understand pathogenesis. Heterozygous mutations in the CSX/NKX2-5 gene have been identified to cause atrial septal defect (ASD) and/or atrioventricular conduction disturbance, characterized by progressive prolongation of the PR interval. There is great variability in expressivity of the phenotype.
We screened 4 sporadic patients and 3 index cases of families with ASD and/or conduction defects. In one of them, a CSX/NKX2-5 mutation was identified. This novel mutation (p.Tyr256X) was inherited in a 3-generation family causing 5 individuals to have cardiac anomalies ranging from ASD to arrhythmias. Surprisingly, no CSX/NKX2-5 mutation was found in the 2 other families presenting a characteristic phenotype of CSX/NKX2-5 mutated individuals. Moreover, an intragenic or whole gene deletion was excluded in one of the families. This suggests genetic heterogeneity for ASD with conduction defects.
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P0870. Association of the serotonin transporter gene (*SLC6A4*) with attention-deficit/hyperactivity disorder in Spanish adult patients

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Attention-deficit/hyperactivity (ADHD) is the most common neuropsychiatric disorder of childhood and it can persist into adolescence and adulthood. Its etiology is multifactorial, with involvement of both genetic and environmental factors. Several lines of evidence suggest a role of the serotonin transporter gene (*SLC6A4*) in the genetic susceptibility to ADHD. Thus, the serotonin transporter has been involved in impulsive behaviour, it plays an important role in the dopaminergic system, which is involved in ADHD, and is a target of different drugs used in the pharmacological treatment of ADHD, such as imipramine or venlafaxine. To evaluate the participation of the *SLC6A4* gene in ADHD we considered the functional 5HTTVNTR polymorphism within intron 3 and performed a case-control association study in a sample of 99 ADHD adult patients and 99 healthy controls matched for age, sex and ethnicity. Although no significant differences were observed in allele frequencies, the genotype distribution of the 5HTTVNTR polymorphism differed significantly between the ADHD sample and the controls (P=0.018), due to an overrepresentation of 5HTT-12R carriers in the group of patients (P=0.009, OR=3.68). We did not detect sex influence, and subdivision of the patients according to the clinical subtypes of inattentive, hyperactive-impulsive or combined ADHD, did not produce any significant association, which could be explained by the relatively small sample size and reduced statistical power. Although preliminary, our results suggest that the 5HTT-12R allele of the 5HTTVNTR polymorphism within the *SLC6A4* gene may predispose to ADHD and support a serotoninergic participation in the etiology of this neuropsychiatric phenotype.

P0871. Identification of gene(s) involved in autosomal recessive autism spectrum disorder (ASD)

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Autism is a complex neurodevelopmental disorder characterized by deficits in social interaction, impaired communication, and unusual and repetitive behaviors and interests. Previous segregation analyses of autism have revealed that some subsets of autism may be caused by autosomal recessive mechanism. Consanguineous pedigrees facilitate the recognition and mapping of recessively inherited neurological disorders. The goal of this study is to use the Arabic Middle Eastern populations to map and identify autosomal recessive autism spectrum disorder (ASD) genes in order to better understand their classification, pathogenesis, and potential treatments. We so far have recruited 65 consanguineous families with autistic children, 20 out of which are multiplex families. SignatureChip Human Genome Microarray were performed on probands from 35 families, and chromosomal anomalies were detected in 6 cases; interestingly, all patients with chromosome deletions or duplications were from families with one affected individual, underscoring the importance of cytogenetic testings in ASD, especially in families with one affected child. Moreover, the male:female ratio in multiplex families was 2.8:1 (37 males:13 females), which appears to be lower than the typical male:female ratio of 4:1. These data support our hypothesis that ASD in some families may be caused by autosomal recessive inheritance. Linkage analyses were conducted in multiplex families. One family maps to the *AUTS1* locus on chromosome 7q, whereas others map to novel autism loci with multipoint LOD score of 2.4-2.78 generated from single families without pooling. The fact that linked loci differ in each family suggests genetic heterogeneity.

P0872. Exclusion of chromosome regions 8p and 8q and 12q in a large Indian family with autosomal dominant Stuttering

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Stuttering (STUT) is an early childhood speech disorder characterized by involuntary syllable repetitions, sound prolongations or interruptions (audible and silent). It begins in childhood between the ages of 3-6. It is a painful symptom and greatly interferes with a child's emotional and psychological development and also affects their daily social or occupational functioning. It has an average prevalence of one in 100 individuals, but varies among various ethnic groups. It affects disproportionately between genders (4:1 male-female ratio). The genes responsible for STUT1 (OMIM 184450) and STUT2 (OMIM 609261) have been mapped to chromosome 18p and proximal 18q (*Am. J. Med. Genet.* 124A:133-135, 2004) and 12q (*Am. J. Hum. Genet.* 76:647-651, 2005), respectively. No causal gene (s) has yet been identified. We have studied a large five-generation Indian pedigree with non-syndromic stuttering in which the anomaly segregates as an autosomal dominant trait. The onset is during early childhood. The pedigrees consist of 68 individuals with 21 affecteds (17 males/4 females). The age distribution of these affecteds is 4-65 years. Severity of the phenotype was variable among affecteds and no skipping of generation was observed. Two-point linkage analysis and haplotype data generated using markers in the known candidate loci on chromosomes 12 and 18 did not show involvement of the above linked regions. Systematic genome-wide linkage analysis, using Affymatrix 10K microchips is in progress to identify the stuttering causing cause gene(s) in this family. Email: u_c_rao@hotmail.com

P0873. Screening of MYO15 gene mutations in DFNB3 locus in autosomal recessive non- syndromic GJB2 and GJB6 negative hearing loss Iranian population

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Background: Mutations in myosin XV are responsible for congenital profound deafness DFNB3 in humans and deafness and vestibular defects in shaker 2 mice. Full-length myosin XV transcript contains 66 exons and encodes 365-kDa protein. MYO15 has at least 50 exons spanning 36 kilobases and is essential for the graded elongation of stereocilia during their functional maturation.Mutations in this gene have been reported in Indonesia, North America and also in our two neighbor countries Pakistan and India; therefore we decided to study this locus in our population.

Materials and methods: Fifty families with autosomal recessive non-syndromic hearing loss with two or more affected children originating from different ethnic groups of Iranian population that were negative for GJB2 and GJB6 mutations, that are located on the most prevalent locus (DFNB1) of hearing loss, were screened for DFNB3 locus by linkage analysis . We used D17S2196, D17S842, D17S1857, D17S2187 and D17S975 STR (short Tandem Repeat) markers for this study.

Results: Two out of fifty families were linked to this locus. Mutation detection of these two families is performing. ***Conclusion:*** We

concluded after DFNB1, DFNB4 and DFNB21 myosin XV gene mutations are responsible for the most prevalent cause of autosomal recessive non- syndromic hearing loss in Iranian population.

P0874. Absence of DFNB9 Locus, as the cause of Non-Syndromic Autosomal Recessive Hearing Loss in the Iranian population

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Inherited hearing impairment affects one in 2000 newborns. Non-syndromic prelingual forms are inherited mainly as autosomal recessive traits, of which 20 genes are currently known . Mutation in the genes that encodes connexin 26 and 30 account for up to 50% of these cases.The gene encoding Otoferlin (OTOF), is responsible for the DFNB9 subtype of prelingual hearing impairment. Different mutations has been reported in the OTOF. So far , mutation in number of regional countries such as Lebanon, Turkey, India and some other European countries like Germany, France, Spain and etc. have been reported. We screened 50 Iranian families with autosomal recessive nonsyndromic sensorinural deafness , which were negative for GJB2 gene mutations. Homozygosity mapping for these families using following STRs markers, D2S1324, D2S174, D2S144, D2S158 and D2S405 was carried out.We have not found any family which could be mapped to DFNB9 in our population. These results suggests that mutation in OTOF gene does not contribute as a major cause of Non-Syndromic heredity hearing loss in our population.

P0875. Polymorphisms in the Glucocorticoid Receptor Gene and Susceptibility to Major Affective Disorders

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A major hypothesis that has been suggested for the pathogenesis of Bipolar and Unipolar disorders is the corticosteroid receptor hypothesis. It focuses on the impaired corticosteroid receptor signaling as a primary factor for the developing of the disorders. For understanding Bipolar disorder, we have to understand the mechanisms that control the range and stability of emotions and it is shown that variations in expression of the glucocorticoid receptor (GR) gene can contribute to the tuning of emotional stability. There is evidence that chronic dysregulation of hypothalamus-pituitary-adrenal axis activity could be associated with the onset and course of Depression.

The GR gene is a member of the steroid receptor family and is located on chromosome 5-5q31.3. In the brain GR is thought to modulate emotional behavior and cognitive functions. We examined whether four polymorphisms (a SNP altering a BclI RFLP, N363S, rs33388 and rs33389) in the GR gene confer susceptibility to Affective Disorders. These SNPs have been shown to be associated with changes in the sensitivity to glucocorticoids.

We studied two large well-diagnosed samples of patients with Bipolar disorder and Major Depression, and matched controls. We did not observe association between developing the diseases and any of the variants. Our results show that none of these polymorphisms exerts a major influence on susceptibility to Bipolar and Unipolar Affective disorders.Currently we are examining the data to study the haplotype pattern, and to test the possible influence of clinical covariates and subtypes

P0876. Branchio-Oto-Renal Syndrome: Identification of a new splice-site mutation in one out of six Danish families

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The Branchio-Oto-Renal (BOR) syndrome is an autosomal dominant developmental disorder characterized by hearing loss and associated branchial and renal anomalies with a prevalence of 1:40.000. BOR is caused by mutations in the EYA1 (Eyes absent homolog) gene (8q13), the SIX1-gene (14q21.3), or in a third locus on 1q31. Six Danish families were studied. In two out of three large families (Fam A and B) linkage analysis showed significant LOD-score to *EYA1* (multipoint LOD score: Fam A: Z = 6.88; Fam B: Z = 5.78), which encodes a transcriptional co-activator with protein-tyrosine phosphatase activity. Sequencing of *EYA1* revealed two splice-site mutations: IVS10-1G>A and ISV12+4A>G, but no abnormalities in the other four probands. The IVS10-1G>A mutation, which is known (http://www.medicine.uiowa.edu), was predicted to move the splice-site one nucleotide and thereby introduce a frame-shift and a premature stop codon at amino acid residue 387. The new splice-site mutation, IVS12+4A>G, co-segregated perfectly with BOR in 12 affected/11 unaffected over four generations and is predicted (https://splice.cmh.edu/) to move the splice-site four nucleotides, leading to a frame-shift and a premature stop codon at amino acid residue 461. Both mutations are predicted to disrupt the hydrolase region of the EYA1 protein.

We identified the disease causing *EYA1* mutations in two out of six Danish BOR families, and identified two splice site mutations, of which one is new. It remains to be investigated if larger genomic rearrangements in *EYA1,* or mutations in other BOR genes/loci can explain BOR in the remaining four families.

P0877. The genetic variation in the C-reactive protein locus has an effect on CVD risk

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C-reactive protein (CRP) is a major acute-phase reactant and an independent predictor of future cardiovascular disease (CVD) events. We analysed two separate, prospectively followed Finnish cohorts, FINRISK 92 and 97, (n=14140) from which 2225 individuals were genotyped using a case-cohort design to study the effect of variation in the CRP gene on the risk of CVD. All haplotype tagging SNPs (htSNPs) with frequency >5% in the SeattleSNP database were analysed.

Four of the six selected htSNPs formed 5 common haplotypes. In time-to-event analysis with Cox's proportional hazard model, two haplotypes showed significant association to CVD events in women. Female carriers of a haplotype tagged by rs3091244(G/A/T)-A had 2.7 times higher risk for CVD event than non-carriers in the FINRISK 92 sample (95% confidence interval:1.0-7.2, p=0.05). In combined analysis of the two cohorts the risk was 2.0 times higher (1.1-3.7, p=0.02). Female carriers of a haplotype tagged by rs2794521(C/T)-C had a significantly lower risk for CVD event in the FINRISK 92 sample (hazard ratio (HR):0.27, 0.1-0.9, p=0.03). The same trend was observed in FINRISK 97 females and in combined analysis for females (HR:0.8, ns. and HR:0.5, p=0.02, respectively). These two haplotypes did not associate to serum CRP concentration in the FINRISK 92 sample and none of the haplotypes associated with CVD risk in males in either cohort.

Variation in the CRP gene may have an effect on the risk of CVD in females as suggested by these results in two independent Finnish population cohorts.

P0878. Androgen receptor gene trinucleotide repeats as a marker for tracing disease in intersex patients

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Mutations of the androgen receptor (*AR*) gene give rise to a wide array of phenotypic abnormalities. Various mutations of the AR gene and expanded CAG repeats at exon 1 of that gene have been reported in patients with infertility and neurodegenerative diseases. However, the role of the AR gene trinucleotides repeats has not been systemically

studied in those with hypospadias or genital ambiguity.
Screening of the AR, LH, SRD5A2, 17 B HSD receptors and SRY gene were carried out in a family with ambiguous genitalia using automated sequence analysis on the ABI Prism 310 machine. Characterization of the 3 patients and their healthy family members did not showed any mutations in the mentioned genes. Analyzing mother and her children AR exon1 showed that mother is heterozygote for both CAG and GGN repeat. All affected children inherited the longer CAG and GGN repeat from their mother and all their healthy siblings inherited shorter CAG and GGN repeat. Only one girl has heterozygous situation like her mother.
Our results indicated that disease locus is in linkage disequilibrium to the CAG and GGN trinucleotide repeats in the AR gene. One of the healthy girls of the family has inherited the expanded repeats which means she is a potential carrier of the disease locus and she will pass the disease locus to her offspring. It is possible to offer her prenatal diagnosis producers. In addition our founding could help the counseling of the affected family member regarding the use of intracytoplasmic sperm injection.

P0879. Evaluating the accuracy of genetic testing for cardiovascular disease susceptibility

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Testing for common disease susceptibility is considered to be of the greatest complexity. However several genetic tests for common diseases are to be appearing in medical practice in few years. While studying the genetic basis for cardiovascular diseases, we detected several genetic polymorphisms as being risk factors for various CVD in Russian population. In particular we had noted that the contribution of genetic polymorphisms to CV endophenotypes interindividual variability could be strictly dependent on the presence of other certain risk factors, such as diabetes mellitus 2 or obesity. For example 'opposite' *NOS3* haplotypes were associated with the presence of left ventricular hypertrophy (LVH) in patients with essential hypertension along and hypertension combined with DM2. So we think that for the aims of susceptibility testing, genetic tests should be combined with the information on CV risk factors existing in patient yet in order to increase test accuracy. To evaluate the usefulness of genetic testing, we studied a sample of 53 males with middle age of 54 years to reveal predisposition to LVH. Test panel consisted of 3 polymorphisms (2 variants in *ACE* and one - in calcineurin A alfa gene, which were associated with LVH in our previous study independently from the other 'classical' risk factors). It was detected that genetic 'diagnosis' (predisposed or not for LVH development) was confirmed by echocardiography in 59%. This was comparable with the share of genetic component, known to be around 60% for this common trait.

P0880. Mutation analyses in families with congenital/infantile cataract

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Families with congenital/infantile cataract (CC) including cataracts associated with micro-cornea (CCMC) were studied to identify cataract-causing mutations. More than 20 cataract genes or loci are known to be associated with either recessive or dominant isolated inherited cataracts but only two genes are known to be related to the association of cataract and micro-cornea.
Materials: Six large families with ADCC and one large ADCCMC family as well as eight small families with ADCCMC are investigated.
Methods: Locus specific haplotyping was done in two of the large families with isolated cataract and complete genome wide linkage analysis was performed in the ADCCMC family. Two point linkage analyses were performed and LOD scores > 3 were obtained. Candidate genes were identified by DNA sequencing using BigDye v1.1 technology and an ABI 377. The identified mutations were further confirmed by diagnostic restriction enzyme digests of the families and 60 normal persons were used as control.
Results: Mutations were identified in three families. A LOD = 3.43 for D1S3466 resulted in identification of the amino acid change S73F, c.218C>T, in exon2 of the gap-junction protein *GJA8* (*CX50*) in one ADCC family. A LOD=2.9 was obtained for D21S1114 close to *CRYAA* in another ADCC family and a mutation R21W, c.61C>T, was detected in exon1. Finally, a LOD = 3.0 was obtained for D21S1114 in the ADCCMC family and a mutation R116H, c.337G>A, was proved in exon3 of *CRYAA*.

P0881. Characterization of two new truncating *PMM2* mutations in CDG-Ia patients: a deep intronic point mutation and a deletion of 20 kb.

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Congenital Disorders of Glycosylation type Ia (CDG-Ia), caused by mutations in *PMM2*, represent the most frequent type of a group of recessive metabolic disorders characterized by a defect in the N-glycan synthesis. The clinical presentation varies between a very severe multisystem disorder to a mild neurological picture. A plethora of PMM2 mutations have been described. The majority of them are of the missense type, reflecting the requirement of a minimal phosphomannomutase activity for a cell or organism to be viable.
We characterized two new truncating mutations in two CDG-Ia patients with an enzymatically confirmed PMM deficiency. Patient 1 is compound heterozygous for the p.V231M (c.691G>A) mutation and a deep intronic point mutation (c.639-15.479C>T). This variant activates a cryptic splice donor in intron 7, resulting in an in-frame insertion of 123 bp between exon 7 and 8. Patient 2 is compound heterozygous for the p.V44A (c.131T>C) mutation and a large deletion of approximately 20 kb including exon 8 of the *PMM2* gene. The transcripts of this truncated allele include exons 1 to 7 from the *PMM2* gene and different fragments of the proximal gene CHSP_1.
These types of mutations have not been described before in CDG-Ia patients and stresses the importance to combine *PMM2* mutation screening on genomic DNA with analysis of the transcripts and/or with the enzymatic analysis of the phosphomannomutase activity. Next to the exonic deletions, which currently receive more attention than before, it is likely that deep intronic mutations represent an increasingly important category of mutations.

P0882. A novel deletion of SATB2 is associated with cleft palate

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De novo translocation interrupting the transcription unit of SATB2 (special AT-rich sequence-binding protein 2) gene located on 2q32-q33 has been reported to be associated with cleft palate only. Mutation analysis of 70 unrelated patients with cleft palate only did not reveal any coding region variants. We tested for the presence of the copy number of SATB2 gene in a sample of 92 patients with cleft palate only using a quantitative real-time PCR approach. In one patient (1%) a de novo SATB2 deletion was detected.
Thus, our study supports the strong evidence of an important role for SATB2 in the palate development.

P0883. Fine mapping of the region on the chromosome 5q32 in Scandinavian families with celiac disease

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We performed a genome wide scan in 106 Scandinavian multiplex families with celiac disease (*Naluai et al, EJHG 2001*). After typing additional markers in the region on chromosome 5q31-33 the NPL

score reached a significant level of 4,2 (p=0,000003). We continued our study by fine mapping a part of this region (approximately 6 cM) between markers D5S2017 and D5S434 in order to perform an association analysis. For this study we analysed 54 SNPs and 8 microsatellites. The SNPs were chosen with consideration to their validation status and position in the region. We focused on possible candidate genes within this region. SNPs chosen had minor allele frequencies above 0,1. The average distance between the markers was approximately 50kb.
The analysis indicated some haplotypes that showed a nominal association (p<0,001). Although promising, it was not significant after correction and could not fully explain the linkage peak, so further investigation of the region is necessary.

P0884. Haplotyping of IVS8 5T-Poly(TG) in CBAVD and pancreatitis patients using a simple allele-specific method

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The IVS8 5T in CFTR gene is an allelic variant of intron 8 that in *trans* with a severe CFTR mutation can result in variable phenotypes, ranging from normal to congenital bilateral absence of vas deferens (CBAVD), or mild cystic fibrosis. Recent studies report a correlation between the exon 9 skipping caused by 5T allele and the adjacent upstream repeat of 9-13 TG. 5T-carrier individuals with either 12 or 13 TG are more likely to exhibit an abnormal phenotype than those with less than 12TG.
We describe here a rapid and simple method for direct haplotyping the TG repeats in 5T individuals by allele-specific fluorescent PCR. 5T-specific primer 5'-CCCCAAATCCCTGTT*AAAAA*C and D4-labelled common primer 5'-GGCCATGTGCTTTTCAAACT are used for PCR amplification. PCR fragments ranging from 120 to 126 bp, corresponding to 10TG-13TG, were detected by automated capillary electrophoresis (CEQ8000, Beckman). Method was assessed in 30 DNA samples carrying 5T allele, in which T-TG tract was previously genotyped by direct sequencing or by cloning into a plasmid vector followed by sequencing in ambiguous cases.
As the method resulted specific, accurate and simple, we further tested the TG repeat in 5T-positive CBAVD and chronic pancreatitis patients, and in normal controls. 100% (7/7) CBAVD, 44,4% (4/9) chronic pancreatitis patients and 48,4% (15/31) normal controls showed 12TG. Our results confirm the pathogenic role of 5T-12TG in CBAVD. Moreover, the high prevalence of 5T-12TG haplotype makes our method an useful tool for a reliable estimation of the disease risk in 5T individuals.

P0885. Is there an association between cystic fibrosis and fatty acid transport protein (FATP) genes?

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Patients with cystic fibrosis (CF) have mutations in the gene coding for the cAMP dependent chloride ion transporting channel in the apical membrane of epithelial cells. There is poor correlation between genotype and phenotype in CF and modifying genes may play a role in disease severity.
Patients with CF have been shown to have abnormal metabolism of fatty acids. The fatty acid transport proteins (FATPs) are involved in the uptake of essential long chain fatty acids in humans and are coded for by 6 known genes, *SLC27A1-6.*
Forty CF-patients, homozygous for the most common severe CF mutation, F508del, and 146 normal healthy blood donor controls with no F508del mutation were analyzed with respect to markers close to the 6 FATP genes to see if there was any association between CF and FATP genes. We used dinucleotide markers from The ABI linkage mapping set, version 2.5. The results were analyzed with the Mann-Whitney test and the two sample Kolmogorov-Smirnov test.
Marker D9S194 showed a week association with SLC27A4 on chromosome 9q31.11. Further testing of markers in the candidate gene in the region will be done. FATP4 is expressed in the pancreas and intestine and has been associated with fasting and postprandial serum lipid levels and insulin resistance. There are also other interesting candidate genes,e.g.CEL (Carboxyl ester lipase) in the region. Mutations in CEL cause pancreatic dysfunction.

P0886. STR panel for PCR-based diagnosis of the CMT 1A duplication in Ukraine

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Charcot-Marie-Tooth neuropathy (CMT) is one of the most common human hereditary disorders affecting 1:2500 individuals. CMT has been separated into two clinical/pathological categories: CMT1 (70%) and CMT2 (30%), which further subdivided according to genetic mapping criteria into -A, -B, -C, and so forth. The major mutation results in CMT1A (68%-90% of CMT1) - is microduplication of 1,4 Mb in 17p11.2 chromosome region. To determine the most informative markers panel of STR-PCR method duplication detection we perform the analysis of polymorphisms in three STR loci (D17S921, D17S1358 and D17S122) from the 17p11.2 chromosomal region in 52 unrelated non-CMT volunteers. The most informative locus was D17S921 with observed index of heterozygosity 73,1%. The overall informativness for the CMT1A-duplication detection in current use STR panel was calculated as 93,6%. Furthermore, the limitations and difficulties in the results interpretation obtained by the currently used method for CMT1A-duplication analysis in Ukraine have been analyzed. We performed the CMT1A tandem duplication analysis using our STR panel for autosome-dominant CMT1 patients and their relatives. CMT1A duplications were found in 29 patients (from 17 unrelated families) of total 21 autosome-dominant CMT families from Ukraine. The patients from 3 CMT families were homozygous in all three loci (non-informative cases). It has been shown that current use STR panel analysis is important for CMT1A duplication detection, early differential diagnosis of CMT including prenatal diagnosis and genetic consulting in high-risk families. However, to increase the informativness of CMT1A-duplication diagnosis new highly polymorphic STR loci have to be involved into analysis.

P0887. Family based association approach provides further evidence for a role of the *CHRM2* gene in cognition

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Identifying genes for variation in the range of normal intelligence could provide important clues to the genetic etiology of disturbed cognition in e.g. autism, reading disorder, and ADHD. Individual performance across a single aspect of cognitive ability is highly predictive of performance on other aspects of cognitive ability.
Cholinergic neurotransmission of muscarinic acetylcholine receptor genes (*CHRM*) has been implicated in higher brain cognitive functions such as attention, learning and memory. The gene encoding mAChR2 (*CHRM2*) on 7q31-35, appears to be predominantly expressed on presynaptic terminals of ACh containing neurons. Pharmacological and electrophysiological studies suggest these receptors serve as autoreceptors, playing a fundamental role in Ach (negative) release regulation.
A family based genetic association test was implemented using quantitative transmission disequilibrium test (QTDT). A sample of 667 individuals from 304 Dutch families was used from which cognition phenotypes were previously collected from several studies from the Netherlands Twin Registry. Three tagging-SNPs (t-SNPs) were selected (rs1174206, rs324640, rs324650). The strongest association, after multiple test correction, was between rs324650 on intron 5 and performance IQ (PIQ), where the T allele was associated with an increase of 4.6 IQ points (*p<0.001)*. We hypothesise that a non-coding polymorphism(s) might be involved in regulation of expression

of alternative splicing of the *CHRM2 gene*, which in turn may affect mAcHR2 transcription, as well as the fine-tuning negative feedback of this particular receptor. Further analyses involving more genetic variants will provide us more insight in order to elucidate the complex interplay among genetic variants and its ensuing consequences.

P0888. Evaluation of Aneuploidy Incidence in Arrested Embryos and Correlation Between Sperm-FISH and Sperm-Apoptosis Results

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Chromosomal abnormality rates in the embryos are observed more frequently than in spontaneous abortions and it has been reported that the incidence of aneuploidies varies between 23% and 89% in embryos in the literature.
In this study we investigated:
1. Chromosomal aneuploidy rate for chromosome 13,16,18,21 and 22 in arrested embryos,
2. The relationship between the aneuploidies found in arrested embryos and viability rate of embryos obtained in the same cycle,
3. The relationship between the aneuploidy rate in arrested embryos and sperm apoptosis, sperm FISH for chromosome X,Y and 18 and spermiogram.
Twenty arrested embryos were analysed using PGD FISH probes (13,16,18,21 and 22). The sperm which was obtained from the father of the arrested embryos was analysed using FISH probes (X,Y and 18). To evaluate apoptosis, TUNEL test was performed on sperm.
Results: Chromosomal aneuploidy frequency in arrested embryos was found to be 80%. There was no relationship between the aneuploidies found in arrested embryos and the viabilty rate of the embryos obtained in the same cycle or total aneuploidy rate in sperm. No correlation was found between sperm apoptosis and chromosomal aneuploidy rates found in arrested embryos. There was a significant correlation between the spermiogram and the number of chromosome aneuploidies in the arrested embryos.
Conclusion: Although there is no significant correlation between the sperm FISH, the sperm apoptosis results and the number of chromosomal aneuploidies in embryos, we propose the spermiogram results are efficacious as an indication of preimplantation genetic diagnosis.

P0889. Analysis of the group-specific component (GC) gene haplotypes in patients with chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is an inflammatory condition of the respiratory system
including partially reversible bronchial obstruction and progressing chronic respiratory insufficiency. Along with the environmental factors, hereditary predisposition is implicated in COPD development. Chronic inflammation plays a special role in COPD pathogenesis. Group specific component is known to bind with vitamin D, extracellular actin and endotoxin. CG enhances the neutrophil chemotactic activity of complement component 5a (C5a) peptide produced during the activation of the complement cascade.
The purpose of this study was to investigate the association of the GC gene haplotypes with COPD. Polymorphisms Glu416Asp and Thr420Lys in the GC gene were investigated in cases of Tatar and Russian COPD patients (N=298) and in cases of ethnically matched healthy individuals (N=237) living in Ufa, Russia.
Analysis of the GC gene polymorphisms in healthy individuals from two ethnic group living in Republic of Bashkortostan revealed statistically significant differences in the haplotypes and phenotypes frequency distributions between Tatars and Russians (χ^2=10.403, P=0.012 and χ^2=14.87, P=0.02, respectively). The GC*1F/1S phenotype of the GC gene is the most widespread in Tatars (36.79%), while the GC*1S/2 phenotype occurs with highest frequency in Russians (33.85%). The GC*2 haplotype was associated with higher risk of COPD in Tatar ethnic group (30.05 vs 18.96% in the control group; χ^2=7.35, P=0.008, OR=1.86 CI 95% 1.17-2.93).
But at the same time we did not find any differences in the haplotypes frequency distributions of the CG gene within the patients and healthy groups in Russian ethnic group.

P0890. Genetic polymorphism in matrix metalloproteinase-9 and COPD severity

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Matrix metalloproteinases (MMPs) are a major group of proteases known to regulate extracellular matrix turnover and so they have been suggested to be important in the process of lung disease associated with tissue remodeling. Increased concentrations of MMP-1 (collagenase) and MMP-9 (gelatinase B) are present in BALF from patients with COPD, and there is increased activity of those enzymes in the lung parenchyma of patients with emphysema.
MMPs are considered as one of the candidate genes in the susceptibility to COPD. A few of naturally occurring polymorphisms of MMP gene promoters have been identified and found to alter transcriptional activity. We determined the prevalence of following polymorphisms: -1607G/GG (MMP1), -1562C/T (MMP9), and -82A/G (MMP12) in 320 COPD patients (II-IV GOLD stages) and 421 healthy subjects to examine the association with the development of pulmonary emphysema. Interestingly, -1562T allele of MMP9 frequency was higher in subjects suffering from very severe COPD (GOLD stage IV) than in patients with stages II and III (0.154 versus 0.085, P=0.011). Association analysis revealed that -1562T allele is a risk factor for development of severe COPD (OR=1.96, CI 95% 1.22-3.34). Moreover, carriers of -1562T allele were more prevalent among stage IV COPD patients younger than 55 years compare to patients with less severe COPD of the same age group (P=0.02; OR=4.22, CI 95% 1.22-15.25).
These results suggest that the polymorphism of MMP9 could be a genetic marker for the development severe clinical course of COPD.

P0891. Cytokine polymorphisms and chronic rejection after organ transplantation

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Graft failure due to chronic rejection, a degenerative process mediated by cytokines and growth factors, remains a significant problem after transplantation: perivascular inflammation, fibrosis and intimal thickening lead to occlusion of graft arteries The implication of cytokines in acute or chronic rejection has been evaluated in several studies. In particular IL10 gene -1082 G/A, -819 C/T, and -592 C/A polymorphisms, TNFalpha -308 G/A and Arg25Pro TGFbeta have been associated with increased levels of heart, kidney or liver transplant rejection. IFNgamma gene 874 T/A polymorphism or IL4R alpha Q576R have been associated with post-transplantation immunological failure. Intercellular adhesion molecule (ICAM)-1 gene E469K polymorphism has been associated with protection from vasculopathy after cardiac transplantation.
The aim of our study is to look for a cytokine polymorphism pattern correlated to risk modification for long term graft failure.
Until now a total of 118 DNA samples from patients with heart and lung transplant rejection (30 heart and 14) or without transplant rejection (48 heart and 26 lung) have been collected.
Eleven gene polymorphisms were analyzed by restriction or by ARMS-PCR analyses: TGF-beta (Arg25Pro, Leu10Pro), IL-10 (-1082 G/A, -819 C/T, -592 C/A), IL-6 (-174 G/C), IFN-gamma (874 T/A), TNF alpha (-308 G/A), IL-4Ralpha (Q576R), ICAM-1 (G241R, E469K).
The distribution of single polymorphism alleles or of three point IL10 haplotypes were not significantly different in transplant patients with or without chronic rejection. This study will be extended to a larger number of patients.Acknowledgements: Italian Ministry of Education, University and Research , COFIN.

P0892. Homozygosity mapping of variant late-infantile neuronal ceroid lipofuscinosis in Turkish families

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Neuronal ceroid lipofuscinoses (NCLs) are a group of autosomal recessive neurodegenerative disorders with a variable age of onset. Within NCLs, the late-infantile onset group (LINCLs) is the most genetically heterogeneous with three genes identified (*CLN2*, *CLN5*, and *CLN6*). A variant form of LINCL (vLINCL) present in Turkish patients (CLN7) has been considered a distinct entity genetically and is clinically characterized by an onset age of 2-7 years, epileptic seizures, myoclonus, psychomotor deterioration, loss of vision, and premature death.

We previously showed that in a subset of Turkish patients vLINCL is caused by mutations in either the *CLN6* or the *CLN8* gene. In eleven families, we excluded by haplotype analysis all known human NCL loci as well as the three loci homologous for genes underlying NCL-like phenotypes in animal models (*CTSD*, *CLCN3*, and *CLCN7*), suggesting that these families represent "true" Turkish vLINCL. We performed a genome-wide scan using 378 microsatellite markers (modified Applied Biosystems LMS-2/MD10 set, Finnish Genome Center, University of Helsinki) in seven of these families, but found no single genomic region showing overlapping homozygosity in all families. Instead, several homozygous regions were observed in a subset of the families suggesting genetic heterogeneity and the existence of more than one NCL-causing, novel gene in the Turkish patients. In order to increase the informativity of the analysis, we undertook a genome-wide scan with Affymetrix GeneChip 50K array. Homozygosity mapping using this high-density genotyping dataset will be performed using GENEHUNTER. We are currently in the process of analyzing the results.

P0893. Large phenotypic variability in 26 novel families with CM-AVM caused by a mutation in the RASA1 gene

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Capillary Malformation-Arteriovenous Malformation (CM-AVM) (MIM 608354) is a recently identified phenotype characterised by atypical capillary malformations (CM) associated with either arteriovenous malformation, arteriovenous fistula, or Parkes Weber syndrome (PWS) (Eerola I., 2003; Boon L.M., 2005). The most prominent feature is the presence of atypical CMs, which are small, round-to-oval in shape and pinkish-red in color. We showed that the CM-AVM is caused by mutations in the RASA1 gene and reported 6 different mutations. The RASA1 gene codes for p120-RasGTPase-activating protein (p120-RasGAP), which is one of the most important inactivators of Ras. Here we report 25 distinct mutations in 26 novel families with CM-AVM. The mutations were spread throughout the gene. Twenty-four mutations caused a premature stop codon: 10 nonsense, 7 frameshift and 7 splicing mutations, suggesting a loss of function as the most likely mechanism. In one family, a missense mutation was identified (Y426C). Sixty-six individuals carrying a RASA1 mutation were identified in these families: 44 had CMs, 20 had CMs plus a high-flow lesion (9 AVM, 1 AVF and 10 PWS) and 2 were nonpenetrant carriers. In summary, 30 different RASA1 mutations have been identified so far, clearly delineating the CM-AVM phenotype. A considerable proportion of patients (30%) had, besides the atypical CMs, a high-flow lesion. Its specific clinical, radiological and genetic characteristics should avoid the confusion with other vascular anomalies, such as hereditary benign telangiectasia or Klippel-Trenaunay syndrome. Our data also suggests that the CM-AVM phenotype could be a relatively frequent genetic disorder.

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P0894. Association of the A1359G polymorphism of the CNR1 gene and central nervous system tumors

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CNR1 gene is located in chromosome 6 and encodes the human cannabinoid receptor CB1. This is a main brain receptor endogenous for anandamina ligans and marijuana constituents, and has a widespread distribution in the central nervous system (CNS). This receptor is implicated in many physiological functions as pain control, anxiety response, movement coordination, corporal temperature regulation, appetite, inflammatory process and addictive development phenomena. CNR1 has a common polymorphism A1359G in third position in its codon 453 which corresponds to a synonymous change in aminoacid Thr. A previous report has linked this polymorphism to brain tumors.

We have analyzed this CNR1 polymorphism in 156 patients diagnosed of glioma and 300 control subjets, both groups aged more than 50. We found a significant difference ($p<0,033$) in allele distribution between glioma patients and controls. Our results suggest that the A allele in CNR1 gene may confer susceptibility for glioma in people aged more than 50 for its significantly association.

P0895. Myosin IXB variant increases the risk of celiac disease and points towards a primary intestinal barrier defect

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Celiac disease has probably become the best-understood immune-related disorder. The disease presents in the small intestine and results from the interplay between multiple genes and gluten, the triggering environmental factor. Although HLA class II genes explain 40% of the heritable risk, non-HLA genes accounting for the majority of the familial clustering have not yet been identified. Here we report significant and replicable association ($P = 2.1 \times 10^{-6}$) to a common variant located in intron 28 of the myosin IXB (*MYO9B*) gene. Homozygosity for the at-risk allele confers a 2.3 higher risk to disease ($P = 1.55 \times 10^{-5}$). Myosin IXB is an unconventional myosin and is expressed in several cells, including leukocytes and epithelial cells. Its Rho-GTPase activating domain can negatively control Rho proteins, which are involved in cytoskeletal modifications and tight junction assembly, suggesting myosin IXB has a role in the epithelial barrier. This may point to a primary impairment of the intestinal epithelial barrier, and may explain why immunogenic gluten peptides are able to pass through this barrier. *MYO9B* could also be an interesting candidate gene for several other disorders since increased permeability of the epithelial barrier is also seen in e.g. inflammatory bowel disorders, type 1 diabetes, and asthma. Alternatively, myosin IXB may play a role in controlling bacterial invasion of epithelial cells and wound closure. We are currently elucidating the exact function of myosin IXB in celiac disease and trying to identify the true functional gene variant that causes the observed genetic association.

P0896. Coeliac Disease susceptibility conferred by DR3/DR3 homozygotes *vs* DR3/DR7 heterozygotes in a Southern European population

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The HLA-associated susceptibility to develop celiac disease (CD) is mainly conferred by the DQA1*05 and DQB1*02 alleles encoding the DQ(α1*05, β1*02) heterodimer (HLA-DQ2). These alleles can be carried in *cis* position on the DR3 haplotype, or in *trans* on the DR3/DR3, DR3/DR7 and DR5/DR7 haplotypes combinations. In Northern Europe DQB1*02-DRB1*03 and DQB1*02-DRB1*07 haplotype frequencies are very similar and they both seem to confer comparable risk to the disease (Louka et al, 2002). In Southern Europe the DR3/DR7 genotype is relatively common while the DR3/DR3 is rarer, and

a higher risk has been proposed for the heterozygotes compared to those DR3 homozygotes.
In order to address this hypothesis, we investigated the effects of different haplotypes carried in trans to the DQA1*05-DQB1*02 haplotype in CD families of Spanish origin, using a previously described statistical method by Louka et al, 2002 (Other Haplotype Test). A sample of 225 affected individuals (112 from Navarra, 80 from Valencia and 33 from Basque Country) who carried at least one DQB1*02-DQA1*05-DRB1*03 haplotype and their parents were included in this study.
We confirmed that DQB1*02-DRB1*03 and DQB1*02-DRB1*07 are strongly associated with CD susceptibility ($p= 5,7x10^{-7}$ and p= 0,001, respectively). However, we found no significant difference in transmisión of one of these haplotypes (p=0,887 and p=0,451). None of the other haplotypes tested showed significant evidence of risk modification (p= 0,17).
Our study shows that DR3/DR3 and DR3/DR7 genoptypes haplotypes confer similar degree of risk predisposition to develop CD in Southern European populations.

P0897. Congenital hip dislocation: a case-control association study for analysing the role of different candidate genes

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Congenital dislocation of the hip (CDH), is a public health matter because of its high frequency, the severe functional handicap induced if it is not treated early and its natural evolution towards hip osteoarthritis. This disease presents a mechanical component linked to the pregnancy and delivery conditions, but the ethnical predispositions and the familial aggregation observed suggest that it also presents a genetic component. We set up an association study in the area of Finistère (western Brittany, France) where CDH is particularly frequent in order to study the implication of candidate genes. To date, 241 CDH patients have been recruited and the cohort is composed of 91.3% of women (n=220 - sex-ratio: 1:10). The pathology was bilateral in 60.0% of cases and when it was unilateral, it affects as often the left than the right hip (48.9 vs 51.1%). Primiparity was observed in 42.3% of cases and breech presentation was documented in 12.4% of patients. In this cohort, 15.0% of the patients had a high birthweight (≥ 4kg) and 5.4% were postmature babies (≥ 42 weeks of gestation). The candidate genes which are studied in first intention include genes involved in the mechanisms of development of members (*HOXB9*) and in the constitution of cartilaginous tissue (*COL1A1* and *COL2A1*). This study will report the preliminary results of the first association study made on CDH and will highlight the role of candidate genes in this complex disease.

P0898. Frequency of *CARD15* Mutations in Patients with Crohn's Disease from Toledo, Spain: Genotype-Phenotype Correlation.

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Crohn's Disease presents a complex multifactorial etiology with genetic and environmental factors implied in the disorder. Epidemiological studies showed that three major *CARD15* mutations have been described as associated to CD: R702W, G908R and 1007fs. We studied frequencies of these three *CARD15* mutations in patients from Toledo, Spain.
One hundred eighty-three patients with Crohn's Disease and one hundred seventy-two healthy controls from Toledo, Spain were included in this study. All of these individuals were genotyped for the three *CARD15* mutations (R702W, G908R and 1007fs). Association analyses to establish genotype-phenotype correlations were performed.
The control population exhibits frequencies of *CARD15* mutations within the range of results of previous studies. Frequencies observed in CD patients were lower than those reported in Caucasian populations; nevertheless we found allelic frequencies to be closer to those observed in some homogenous populations: 0.076, 0.030 and 0.046 for the R702W, G908R and 1007fs mutations, respectively. Significant associations were found for the R702W and for patients carrying two mutations to an early age of onset and stricturing pattern. Additionally, the presence of at least one mutation was correlated to the disease.
The presence in heterozygosity of R702W mutation, or two *CARD15* mutations is associated to an early age of onset and stricturing pattern, while patients with at least one mutation presented association with stricturing and ileal form. We observed that frequencies of these groups were lower than in Caucasian populations, although similar ranges of frequencies were observed to those in homogeneous populations.

P0899. Frequency of double mutant alleles in cystic fibrosis patients from Serbia and Montenegro

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Cystic fibrosis (CF) is the most common autosome recessive disease in Caucasians.More then 1300 mutations in CF transmembrane conductance regulator (CFTR) gene are causative for both classic and atypical presentations of the disease.Complex alleles with more then one mutations *in cis* are rare,but observed in some populations.
Since 1995.,in order to confirm the clinical diagnosis of CF,we have screened 179 patients for molecular defects in CFTR gene. A total of 18 mutations covered 82.41% of CF alleles,with F508del showing a frequency of 72.35%.For almost 30% of the CF alleles (51 patient),mutation screening covered the entire coding region using DGGE and sequencing of PCR-amplified genomic DNA.As a result,we identified double mutant alleles in 2 patients of those investigated (2/51).Both patients were compound heterozygous for F508del inherited from one parent,and S466X with R1070Q *in cis*, inherited from the other parent.Patients were unrelated but from the same region of our country,which suggest that their families might have common ancestor from whom they inherited this unusual allele.Patients were females,with similar clinical symptoms;7 years old,age at diagnosis before first year of life.Chloride sweat values were more then 91mmol/L,they were PI since birth,with obstructive lung disease and chronic cough.In both cases phenotype was severe despite the fact that one of the mutations (R1070Q) belongs to class IV (mild mutations).
We will discuss if these results suggest that double mutant alleles might be more common than expected and therefore have important implications for molecular diagnosis and genetic counseling of families at risk in our country.

P0900. The 8.1 ancestral haplotype is associated with delayed onset of colonization in cystic fibrosis

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Rationale: Major cause of death in patients with cystic fibrosis (CF) is colonization with *S. aureus* and *P. aeruginosa*. The wide phenotypic variation in CF patients -with similar genetic background- suggests that genes other than the cystic fibrosis transmembrane conductance regulatory *(CFTR)* gene modify the disease. The 8.1 ancestral haplotype (8.1 AH) in main histocompatibility complex is associated with alterations of the immune response.
Objective: To study the influence of carriage of 8.1 AH on frequency and onset of colonization in CF patients.
Study population, methods: DNA samples of 72 CF patients (39 homozygous and 33 heterozygous for the ΔF508 mutation of *CFTR*

gene) were genotyped for the member alleles of the 8.1 AH: *HLA-DQB1*0201, HLA-DRB1 *0301, RAGE -429C, HSP70-2 -1267G* and *TNF-α -308A*. Patients were recruited from seven Hungarian CF centers. Colonization was verified by regular clinical and bacteriological screening.
Findings: Frequency of colonization was significantly (p=0.0002) lower in the 8.1 AH carriers; age, gender and ΔF508 genotype-adjusted odds ratio to be colonized of the carriers vs. non-carriers was 0.04 (0.006-0.29). Patients with 8.1 AH had significantly (p=0.0002) longer colonization free period compared to non-carriers. **Conclusion:** Our novel observations demonstrate that the 8.1 AH is associated with delayed onset of colonization in CF, presumably by influencing defense mechanisms against infections.

P0901. Beta 2 adrenergic receptor polymorphisms in Cystic Fibrosis liver disease

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Cystic fibrosis (CF; #219700) is a disorder caused by mutations in the CFTR gene that encodes a chloride conducting channel expressed in the apical plasma membrane of the epithelial cells. The disease is characterized by a wide variability of clinical expressions which include pancreatic insufficiency, lung disease, hepatic manifestations, and male infertility. It is possible that the wide phenotypic spectrum is modulated by "modifier genes" which directly or indirectly interact with CFTR gene product. Recently, biochemical and functional analysis of CFTR, revealed that this protein interacts with protein containing multiple PDZ domain. We studied beta-2-adrenergic receptor (ADRB2), a protein containing a PDZ domain, belonging to the G protein-coupled receptor superfamily and expressed in different tissues including the apical membrane of the epithelial cells. We genotyped 52 CF patients (E) with hepatic involvement, carrying two class I mutations, homozygous F508del, or compound heterozigous for a class I allele and F508del. We selected as control subject 47 CF patients without hepatic involvement (CFC) and 30 unaffected individulas (C).
We genotyped three polymorphisms in the ADRB2 gene, -47C/T, Arg16/Gly and Gln27/Glu and derived haplotypes. We observed that haplotype -47C-Gly16--Glu27 is differently distributed between CF classes (E= 21,5% vs. CFC= 2,7%) p=0,001. This haplotype is present in the normal population with a frequency of 18.1%.
Though the number of CF patients is small, and these data need to be confirmed in larger studies, they suggest that the ADRB2 gene might be a modifier gene for liver disease in CF .

P0902. Sequence analysis of *CFTR* coding region in Croatian population

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Cystic fibrosis is one of the most common recessive disorders in Europe affecting approximately 1 in 3 000 individuals. More than 1200 mutations have been identified in cystic fibrosis transmembrane conductance regulator gene (*CFTR*). The most frequent mutation, accounted for about 67% of CF chromosomes, is ΔF508. Only four other mutations (G542X, N1303K, G551D and W1282X) have overall frequencies higher than 1%; most other are rare and specific for some population subgroups. The aim of this study was to reveal the frequency of CF mutations by sequencing analysis of coding region. Our previous results by INNO-LIPA CFTR detection kits on total of 41 unrelated CF patients from Croatia revealed only 6 different mutations accounted for 68,29% diseased alleles. Here we analyzed only those samples from previous study that were wild type homozygous or mutated heterozygous. We found three new mutations, so far not detected in our population: S466X (exon 10), Y569C (exon 12) and E585X (exon 12).

P0903. Genetic polymorphism of *CYP1A1*, *GSTM1* and *CYP2C9* is associated with susceptibility to non-Hodgkin's lymphoma and chronic lymphocytic leukemia in adult Russian patients.

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At present the association of different polymorphic variants of biotransformation system genes with the increased risk to develop oncological diseases is investigated actively. We developed a biological microchip which allows to analyze 18 mutations in eight genes of biotransformation system: *CYP1A1*, *CYP2D6*, *GSTT1*, *GSTM1*, *MTHFR*, *MTRR*, *NQO1*, *CYP2C9*, *CYP2C19* and *NAT2*. This biochip has been used to study 76 non-Hodgkin's lymphoma patients (NHL), 83 chronic lymphocytic leukemia patients (CLL) and 177 healthy control subjects. It was found that individuals carrying heterozygous and homozygous variants of *CYP1A1* gene showed statistically significant increased CLL risk: for 4889A>G and 6235T>C (OR = 1.75, p = 0.03); for 4887C>A, 4889A>G and 6235T>C (OR = 1.69, p = 0.02). *GSTM1* null genotype individuals showed a 1.8-fold increased NHL risk (p = 0.04). In combined results, subjects with the „unfavorable" polymorphic variants of *CYP1A1* gene and *GSTM1* null genotype revealed statistically significant increased CLL risk: for combination with heterozygous and homozygous variants 6235T>C (OR = 2.42, p = 0.02); with polymorphic variants 4889A>G and 6235T>C (OR = 2.24, p = 0.007); with polymorphic variants 4887C>A, 4889A>G and 6235T>C (OR = 2.20, p = 0.005). Besides it was obtained that men carrying heterozygous and homozygous variants gene *CYP2C9* showed statistically significant increased CLL risk: for 430C>T (OR = 2.7, p = 0.026); for 430C>T and 1075A>C (OR = 2.1, p = 0.02). Our data demonstrate that polymorphic alleles of genes *CYP1A1*, *GSTM1* and *CYP2C9* might increase the risk of NHL and CLL in the Russian population.

P0904. Number of deafness genes in Israel: up to 8

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Hearing loss (HL) is a highly heterogeneous genetic disorder, with over 100 genes predicted to be involved. Until recently, mutations in four genes were known to lead to non-syndromic HL (NSHL) in the Israeli population. These include *GJB2*, *GJB6*, *MYO3A*, and *POU4F3.* Of these, *GJB2* mutations are the most common, associated with 39% of children born with HL. Loci for 2 more genes for deafness have recently been mapped in Israeli families with otosclerosis (*OTSC4*) and progressive NSHL (*DFNA51*).
Mutations in *GJB2* and *GJB6* cause congenital recessive SNHL, usually severe to profound. Three different mutations in *MYO3A* are responsible for progressive late onset high tone SNHL, inherited in a recessive mode. A dominant *POU4F3* mutation leads to late onset progressive HL. We have recently found that a *SLC26A4* mutation is involved in post lingual progressive recessive HL accompanied with an enlarged vestibular aqueduct. We also found that a *CDH23* mutation is associated with congenital profound recessive deafness.
For syndromic HL, mutations causing USH1, USH2, and USH3 have been found in Israel, all involving combined deafness and blindness due to retinitis pigmentosa. These include mutations in the *USH2A* gene in Iranian and Moroccan Jews and the N48K *USH3A* mutation in Jewish Ashkenazi individuals with postlingual progressive HL. The R245X mutation of the *PCDH15* gene causing USH1F was identified only in Ashkenazi Jews,.
It has been estimated that there are 8-9 genes in Israel for recessive HL, and attempts to identify all the deafness genes in this population are underway.

P0905. Angiotensinogen M235T polymorphism is associated with symptoms of depression in a population-based study and a family-based study

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Objective- Although several lines of evidence suggest that angiotensinogen (AGT) is involved in depression, no study has considered the relation between the AGT gene and unipolar depression. The present study examines the association between the AGT M235T polymorphism and symptoms of depression in two independent populations. *Method*- Cross-sectional data from two independent Dutch cohorts were used for the analyses: 1) The Rotterdam Study, a population-based study among 7,983 individuals 55 years and older in an outbred population, and 2) the Erasmus Rucphen Family Study (ERF), a family-based study of 2,727 relatives from a genetically isolated village. Symptoms of depression were assessed with the Centre of Epidemiological Studies Depression Scale (CES-D). We compared mean CES-D scores between the MM, MT, and TT genotypes of the AGT M235T polymorphism. In the family-based study we additionally calculated to which extent AGT M235T explains the heritability of the CES-D scores by performing a variance components analysis. *Results*- In both populations we found a significant relation between the AGT M235T polymorphism and CES-D scores in men. In ERF the heritability estimate for CES-D scores was 32%. When AGT genotype status was included in the analysis the heritability decreased to 31%. The AGT genotype contributed to 1% of the total variance in the CES-D scores. *Conclusion*- Our findings suggest that the AGT is a susceptibility gene for symptoms of depression. Since the association was only seen in men, different pathways in depression may be involved in men and women.

P0906. Hypertension genes are genetic markers for vascular complications in type 2 diabetes

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Type 2 diabetes often lead to vascular complications. Several genes involved in salt sensitivity and in the renin-angiotensin system (RAS) play a role in the regulation of blood pressure and vascular pathology. We examined the association between alpha-adducin 1 gene (ADD1) Gly460Trp polymorphism, angiotensinogen gene (AGT) M235T polymorphism and the angiotensin II type 1 receptor gene (AT1R) C573T polymorphism with vascular complications and mortality in type 2 diabetes. The study was part of the Rotterdam Study, a population-based cohort study of 7983 subjects aged > 55 years. For ADD1, we observed that TT carriers had both a higher risk of hypertension (RR = 2.57, 95% CI: 1.05 - 6.32), and high levels of atherosclerosis as measured by the mean common carotid intima media thickness (IMT) (p trend = 0.03). The risk of mortality in diabetic patients with hypertension was higher in TT carriers compared to the GG carriers (RR = 1.83, 95% CI: 1.07 - 3.16). For AGT, carriers of the T allele had significantly higher mean systolic and diastolic blood pressure (p = 0.01, p = 0.05), a higher risk of hypertension (RR = 1.85, 95% CI: 1.28 - 3.67), and a higher risk of carotid artery plaque (RR = 1.57, 95% CI: 1.04 - 2.38). Furthermore, TT carriers of AT1R had a significantly increased mean carotid IMT (p = 0.04) compared to the CC genotype. Our results suggest that the genes involved in salt sensitivity may be important determinant of vascular complications in patients with type 2 diabetes.

P0907. Lipid profile and glucose metabolism in patients of type 2 diabetes mellitus in relation to family history

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In patients of type 2 Diabetes Mellitus (T2DM) the process of atherosclerosis is accelerated. Presence of positive family history of type 2 diabetes mellitus is associated with adverse changes in lipoprotein and glucose metabolism of nondiabetic patients.
Our study aimed to compare anthropometric data, lipid and glucose metabolism and inflammatory factors in T2DM patients with positive family history [Fam (+)] and negative family history [Fam(-)] of T2DM. We are studied 22 T2DM patients whose first degree relatives suffered from T2DM and 33 patients without T2DM among close relatives.
Methods. Weight, height, waist and hip circumference were determined, body mass index and waist to hip ratio were calculated. Fasting serum glucose and insulin were measured and used for calculation of insulin resistance index by homeostasis model assessment (HOMA IR). Lipoprotein parameters (TC, TG, HDLC,) were determined enzymatically, C-reactive protein by a highly sensitive immunoprecipitation test and fibrinogen by the method of Clauss.
Results. In the Fam(+) male patients T2DM was diagnosed significantly earlier than in Fam (-) (49.27±5.27 yr and 52.33±0.98 yr, respectively). Fam (+) males with T2DM had also three times more frequently cardiovascular complications than the Fam(-) patients. Serum glucose level of Fam(+) male T2DM subjects was marginally higher as compared to Fam(-) male patients. Lipoprotein parameters and inflammatory markers did not differ between the studied groups.
Conclusion. Preliminary data showed that positive family history has certain relation to some studied indices in male T2DM patients, the dependence was not noticed in females.

P0908. Susceptibility to Type 1 Diabetes Mellitus and Type 2 Diabetes Mellitus related with polymorphisms located in IDDM2 region

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Contribution of insulin (-23Hph, +1127Pst) and IGF2 (IGF2Apa) gene polymorphisms are tested for association with Type 1 Diabetes Mellitus (T1DM) and Type 2 Diabetes Mellitus (T2DM).
The aim of this case-control study was to analyze the role of IDDM2 polymorphisms to the T1DM and T2DM susceptibility.
Clinical information and biological samples from 400 unrelated Romanian Caucasian subjects were collected. They are fall into four equal groups: T1DM (men: women, 53:47, age 34±7 year, duration of T1DM 19±8 year), control T1DM (fasting glicemia 97±9 mg/dl), T2DM (men: women, 59:41, age 55,7±8,1 year, duration of T2DM 13±8,4 year), control T2DM (fasting glicemia 93±6 mg/dl). The control T1DM and control T2DM groups were matched for both age and gender with T1DM and T2DM groups respectively.
The -23Hph, +1127Pst and IGF2Apa polymorphisms were genotyped by PCR-RFLP.
The distribution of genotypes in all groups was in agreement with Hardy-Weinberg equilibrium.
In addition, the reduce number of recombination between -23Hph and +1127Pst sustains the strong linkage between these polymorphisms.
We found a strong association between -23Hph +/+ (95%CI; 1,8664<OR<6,408; p<0,0001) and +1127Pst-/- (95%CI; 1,9429<OR<6,7237; p<0,0001) genotypes and T1DM development. This result is very similar with those published in the literature. The distribution of IGF2Apa genotypes do not differs significant between T1DM and control T1DM (p>0,05). Comparing with other Caucasian population, no difference in alleles or genotypes frequencies of analyzed polymorphisms between T2DM and control T2DM has been observed. This result could represent a feature of Romanian population or a bias caused by study design.

P0909. Analysis of SNP polymorphisms in IDE gene in patients with type 2 diabetes mellitus from Russia

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The aetiology of complex type 2 diabetes mellitus (T2DM) involves both environmental and genetic factors. Insulin degrading enzyme (IDE) gene represents a strong positional and biological candidate for T2DM

susceptibility. IDE is the Zn requiring metalloproteinase, which plays a principal role in the insulin degradation. The linkage to chromosome 10q23-q25 (where IDE gene maps) was identified for T2DM and related quantitative traits. IDE knockout mice are characterized by decrease insulin degradation, hyperinsulinemia, glucose intolerance. It is interesting that significant evidence are demonstrated for effects of sequence variations in IDE on both T2DM and plasma insulin and glucose levels in some human populations of European descent, whereas such associations are not shown in other populations. To evaluate a role of IDE gene polymorphisms in T2DM development in Russia, we studied 5 SNPs within the IDE gene and in its region in 99 patients with T2DM and 102 controls. An initial case-control analysis revealed no significant differences in distribution of genotypes and alleles between groups of diabetic and control subjects. We found no associations between studied sequence variations and T2DM risk. We can make a preliminary conclusion that analysis of Russian population provides no evidence of the contribution of IDE polymorphisms to T2DM susceptibility.

P0910. Genomewide linkage of a large Korean family with dilated cardiomyopathy

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AIMS: Dilated cardiomyopathy is a disorder characterized by cardiac dilation and systolic dysfunction. So far sixteen genes have been shown to cause autosomal dominant familial dilated cardiomyopathy. We identified a large Korean family from the Cheju Island showing a clear dominant Mendelian inheritance pattern of dilated cardiomyopathy and we proceeded to locate the underlying gene in this family by using genetic linkage.

METHODS: Twenty nine members of this four generation pedigree were recruited, including DNA from six clearly affected individuals. The diagnosis of dilated cardiomyopathy was made by our group according to standard criteria. Genomic DNA was isolated from peripheral blood samples. 392 microsatellite markers at an average of 9 cM distance were genotyped throughout the genome. We used a dominant genetic model with 0.95 penetrance, 0.001 phenocopy rate, and a disease allele frequency of 0.001. Individuals younger than 40 years of age were set to undetermined status for the analyses.

RESULTS: Five regions with LOD scores above 1 were found in parametric two-point linkage analysis on chromosomes 1, 2, 7, 13 and 21. Linkage to D1S1595 was strongly supported by the multipoint linkage analysis with a LOD=2.82, the highest score in the study. Fifteen additional markers were added in the D1S1675 to D1S2107 interval. Haplotype analysis with the additional markers allowed the definition of a 27 cM candidate interval (45.9 Mb) including the centromere and flanked by markers D1S1675 and D1S104.

CONCLUSIONS: The interval contains the known candidate gene LMNA encoding the laminin A/C which we will pursue.

P0911. A novel locus for dilated cardiomyopathy, diffuse myocardial fibrosis and sudden death on chromosome 10q25-q26

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Background: Genetic studies have mapped multiple loci for inherited dilated cardiomyopathy (DCM), a major cause of non-ischemic heart failure. However, the mutated genes responsible for the majority of cases have yet to be been identified.

Methods: DCM was diagnosed on echocardiography if the left ventricular end diastolic dimension was > 117% of normal when corrected for body surface area and age and there was evidence of impaired contractility i.e. left ventricular ejection fraction was < 0.50, or fractional shortening was ≤ 28%. Genome-wide linkage analysis was conducted using a 10 centimorgan resolution panel of microsatellite markers.

Results: We have identified two kindreds, CM-50 and CM-100 in which early-onset DCM segregates as an autosomal dominant trait. Clinical characteristics of this unusual form of DCM were sudden cardiac death (SCD) and severe diffuse myocardial fibrosis. In the two families a total of 32 individuals were considered affected, 25 unaffected and 9 of unknown status. Peak two point LOD scores >3.0 were obtained independently with each family using the markers D10S1773 and D10S1483 respectively. Haplotype analyses in the two families identified substantial locus overlap, and when considered together, defined a critical interval of 14.0 centimorgans between D10S1237 and D10S1723. Multipoint linkage analyses confirmed this interval and generated a peak LOD score of 8.2.

Conclusions: We have mapped a novel locus for cardiomyopathy, diffuse myocardial fibrosis and SCD to chromosome 10q25-q26. Identification of the causative gene will be an important step in understanding the fundamental mechanisms of heart failure and sudden death.

P0912. Characterization of dinucleotide bands to differentiate between homozygotes and heterozygotes in diagnosis analysis

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Backgrounds: Microsatellites are sequences made up of single repeats (1-6 bp) which are repeated side by side through out the human genome. Short tandem repeats (STRs) are powerful genetic tools for genetic mapping and could be used for human identification due to their high level of polymorphisms. Among STRs, dinucleotides are more difficult to type. Here in, dinucleotide repeats were used for diagnostic purposes.

Methods: DNA amplification of two repetitive regions of factor VIII gene of 70 unrelated individuals was performed. The PCR products were detected on 10% polyacrylamide gel by silver nitrate staining. Many bands were seen when working with dinucleotides which led us to genotyping errors. For characterizing each band, they were recovered from the gel. The recovered bands were cloned for sequencing analysis and each band was characterized.

Results: Detected bands were the result of errors produced during PCR processing. Allelic bands were the ones with stronger intensity. Minor bands were also seen including stutter bands, shadow bands, non-temple products, microvariety bands, and bands formed by the mutations in the repetitive region itself. Also heteroduplex and conformational bands were the result of electrophoresis conditions.

Conclusions: STR markers allow rapid testing of carrier detection, prenatal diagnosis and genetic transmission Studies. Therefore indicating the errors are important for defining the true allele and genotyping the population. We have concluded that these definitions were effective for detecting PCR processing errors in studying dinucleotide repeats that would lead to mistyping of heterozygotes and homozygotes.

P0913. Dopamine D2 receptor gene polymorphisms and their contribution to personality traits

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The heritability of human personality traits has been estimated between 30 and 60%, based on data from twin and adoption studies. It has been demonstrated that common genetic polymorphisms in the dopamine D2 receptor gene (*DRD2*) have been associated with Novelty Seeking and other personality traits characterized by detached, schizoid, or avoidant behaviour.

In this study healthy Russian subjects (n=287) who administered Cattel's 16PF Questionnaire (16PF CPQ) and Eysenck Personality Questionnaire (EPQ) were analyzed with regard to the *DRD2 TaqIA* and *NcoI* polymorphisms. We observed significant association between *DRD2*N1* allele and Rule-consciousness (F=4.509, p=0.035) score, and between *DRD2*N2* allele and Vigilance score (F=4.078, P=0.044) of 16PF CPQ. Using GLM we found statistically significant correlations between the presence of **A1* allele of *TaqIA* polymorphism

and gender with Self-reliance of 16PF CPQ (F=4.298, p=0.039). There were tendencies toward association between the *DRD2*A2* allele and Openness to change (F=4.499, p=0.035) and Privateness (F=4.638, p=0.032) scores when gender was entered as the second factor. In contrast, one-way ANOVA didn't reveal any differences among the two groups - **N1*-presense versus **N1*-absence or **N2*-presence versus **N2*-absence allele carriers of *NcoI* polymorphism - in any of the EPQ and CPQ personality dimensions. According to QTLs approach increased level of significance can be revealed whether combined effect of polymorphic variants of genes coding for components of neurotransmitter systems of brain are taken into the consideration.

P0914. Electrocardiograms in Down syndrome patients: siblings control

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Chromosome syndromes are polymorphic because of chromosome imbalance lowering the threshold of appearance for inherited multifactorial traits. This means that these traits could be inherited in chromosome patients from their parents more frequently than in their siblings. According to this hypothesis we investigated electrocardiograms (ECG) in Down syndrome (DS) patients. The single parameter of ECG (individual peak amplitude, the duration of it and interval between it) *per se* is the final reflection of multiple hereditary components beginning since histogenesis.

The ECG's were studied in a group of 302 patients with cytogenetically proven trisomy-21. For all of them typical 12 standard leads were taken in region hospitals. The age of patients ranged in the limits from 1 to 46 yrs (average age 15.3 yrs). It seems that for such heterogenic contingent it is difficult to choose adequate control group, especially having in mind the fact that in hospitals and outpatient departments ECG is recorded when some heart pathology is suspected. In all aspects, in our opinion, siblings are the best for control. ECG's were taken for 278 brothers and sisters (age interval ranged from 1 to 60 yrs, average age 20.0 yrs). The ECG anomalies were divided up into 15 classes following manifestations usual for physicians. Pathology in ECG was found in 173 (57.0%) DS patients and in 108 (38.9%) of their siblings. The most frequent ECG pathology in DS patients is the bundle branch blocks ($p<0.01$) and the ventricle preexcitation (short PQ and WPW syndrome) is possible.

P0915. Mutant DMD allele carried by different maternal X chromosomes in two brothers

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Segregation analysis of chromosomes carried by affected individual is a helpful approach for genetic counseling when the mutation itself is not identifiable. The interpretation of the finding, however, may be erroneous, since the mutant allele could be transmitted from one chromosome to the other by recombination event during homologous pairing, as the case presented indicates.

In two brothers Duchenne muscular dystrophy was proved by mutation analysis of the dystrophin (DMD) gene showing deletion of exons 45-50. We performed microsatellite marker analysis of the X chromosomes in the family, in order to identify the X chromosome carrying the mutation in the obligate carrier mother. The markers in (5' DYS MSA, 3' DYS MS, IVS44SK21, DMD-45, DMD-49, DMD-50, DysI, DysII, STR-44, STR-45, STR-49, STR-50) and around (DXS451) the DMD gene showed that the two brothers have, as expected, identical X chromosomal region. Surprisingly, however, markers on the distal short and long arms (DXS207, DXS691, DXS1283E) could be assigned to different maternal X chromosomes in the two sons, indicating that the DMD gene carrying the deletion might have been transferred between the two maternal X chromosomes.

Moreover, further extended marker analyses of samples taken from the maternal grandparents and other members of the mother's family suggested germinal mosaicism of the mother. Our case shows that well-constructed microsatellite marker analyses, in addition to help genetic counseling, may shed lights on the nature and mechanism of disease causing mutations as well.

P0916. Parkin mutations in patients with early-onset parkinsonism

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Parkin gene mutations are reported to be a major cause of early-onset parkinsonism (age at onset <45 years) in families with autosomal recessive inheritance and in isolated juvenile-onset parkinsonism (age at onset <20 years). The mutations in the parkin gene are also extremely varied and include many different point mutations and exonic rearrangements affecting all 12 of the coding exons. To evaluate the frequency of parkin mutations in patients with isolated early-onset parkinsonism, we studied 61 patients selected with an age at onset <45 years from southern Italy, by conventional and quantitative polymerase chain reaction. Among the 61 patients with early-onset parkinsonism but without family story, 13 (21.3%) had mutations in the parkin gene: 7 carried single heterozygous mutations (Thr55Ile, Asp18Asn, Lys32Thr, Asp86Asn, Met192Leu, Leu261Leu, Arg42Pro); 1 was compound heterozygous (Leu174Leu, Arg402Cys); 5 carried simple homozygous mutations (Arg42Pro, 202-203delAG, ex2-3 del, ex3 del). 5 of these mutations were not previously described (Thr55Ile, Asp18Asn, Lys32Thr, Asp86Asn, Leu174Leu). The majority of mutations detected in this study affected the ubiquitin domain and the C-terminal two ring finger domains (R1-R2), which probably resulted in a loss of function of the parkin protein. The role of heterozygous parkin mutations as potential PD susceptibility factor remains to be determined.

P0917. Serotonin genes and their role in eating disorder behaviours

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Eating disorders (ED), e.g. anorexia and bulimia nervosa, are characterized by severely disordered eating behaviour, mainly affecting young women. Despite substantial efforts to identify causal pathways for ED, very little is known about the aetiology of these disorders. In a pilot sample of adolescent twins (N=579) and their non-twin siblings (N=142) of the Netherlands Twin Registry a factor analytic approach was used on the items assessing ED behaviours and traits such as, dieting, fear of weight gain, and importance of body weight or shape on self-evaluation to define a broad ED phenotype. The majority of these items could be explained by one underlying factor representing a quantitative measure of such a broad ED phenotype. Using the twin-sibling design it was subsequently indicated that individual differences in this factor are mainly explained by an underlying genetic mechanism in females.

In the current study we report on whether genes involved in the serotonin pathway contribute to the broad ED phenotype. We collected items describing the broad ED phenotype in a larger sample of adolescent twins and their siblings (questionnaires were sent to 2000 families) as part of the Dutch Health Behaviour Questionnaire, DNA samples are available for 241 MZ twins and 155 DZ twin pairs. SNPs within the serotonin receptor 1A (n=3), 1D (n=4), 2A (n=3) and 2C (n=1) genes are measured and currently analysed .We will present results of the family based haplotype association analyses for these genes and the broad ED phenotype in this sample.

P0918. Mutational analysis of EFHC1 gene in Italy families with Juvenile Myoclonic Epilepsy

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Juvenile myoclonic epilepsy (JME) is a common form of generalized epilepsy starting in adolescence. Recently, a major JME locus was mapped to chromosomal region 6p12.p11 and it was associated with mutations in the EFHC1 gene. This gene contains 11 exons and encodes a protein of 640-amino acids that contains 3 DM10 domains and an EF hand calcium-binding motif.

In this study, we screened for mutations in the EFHC1 gene 25 families from Italy in which at least two members had a typical form of JME.

25 families were selected and families with fewer than two affected members were excluded from the study. In each patient the diagnosis of JME was done according to the ILAE criteria. After informed consent, DNA was isolated from peripheral blood lymphocytes by standard methods and each exon of EFHC1 gene was amplified and sequenced using intronic primers.

We have identified three heterozygous mutations: the F229L mutation, previously described, and the novel mutations P429P and R353W among affected members of 4 unrelated families.

EFHC1 gene has been associated with JME in six out 44 families from Belize, Los Angeles, and Mexico. The authors detected 3 heterozygous mutations (F229L, D210L, D253Y) and one double heterozygous mutation (P77T, R221H). The results of our study are important as they extend for the first time the distribution of EFHC1 mutations to Caucasian populations. Moreover, our data provide further evidence for the high level of genetic heterogeneity associated with JME, as most of our JME families did not carry any mutations.

P0919. An early-onset progressive encephalopathy with myoclonus and dystonia (PEMD) mapping to chromosome 16pter

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We describe a novel recessive myoclonic encephalopathy characterized by very early onset and a steady progressive course. In addition to various types of myoclonic seizures, clinical features include episodic phenomena as dystonia, alternating, post-ictal enduring hemipareses, autonomic involvements and periods of obtundation and lethargy. Developmental and neurological retardation, accelerated with systemic infections, lead to a full deterioration. We present two subjects in a Turkish inbred community. The onset is within 2 months after birth, and death occurs within eight years. We designated the disease progressive encephalopathy with myoclonus and dystonia (PEMD). A genome-wide scan for the family and subsequent fine mapping localized the gene responsible for the disease to the 5.98 Mega base (10.36 cM) p-terminus of chromosome 16. The maximum multi-point logarithm of odds score 4.338 was obtained around D16S3124. Several genes map to the gene locus, including SLC9A3R2, SYNGR3, SSTR5 and ATP6V0C. Other metabolic genes mapping to the gene locus will also be discussed. Although the molecular basis for this new encephalopathy with predominantly myoclonic and dystonic features remains unidentified, the localization of a gene responsible for the novel disorder may have benefits for families afflicted with diseases exhibiting similar clinical features, as they can be tested for linkage to the locus.

P0920. Analysis of polymorphisms metabolisms genes (CYP19, GSTM1, GSTT1 and NAT2) in endometriosis patients with different efficiency of hormone-modulate therapy.

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Endometriosis is an estrogen-dependent disease of women of reproductive age. The main direction of conservative therapy of endometriosis patients is the recovery of unstable hormone status with anti-estrogens drugs. However, some patients are resistant for hormone-modulate therapy and have severe side effects. Retrospective analysis of the results of hormone therapy allowed to divide patients to two groups according to hormone therapy efficiency. Group 1(54 patients) revealed positive responses to combined surgical and therapeutic treatment. Some women have demonstrated conspicuous resistance to this kind of treatment and were arbitrarily attributed to group 2 (63 patients). An increased frequency of A1 allele of the CYP19 gene was observed in group 2 as compared to group 1 (32% versus 17%, p <0.01). Carriers of the A1/A6 genotype were registered in 30.6% of group 2 as compared to 4.7% in group 1 (p <0.01). Analysis of combination of functionally impaired alleles of the GSTM1, GSTT1 and NAT2 revealed significant difference between two groups of patients. The combination of the GSTT 0/0 and NAT2 S/S (p<0.05; OR 8.3), GSTM1 0/0 and NAT2 S/S (p<0.01; OR 8.6), GSTM1 0/0 and GSTT1 0/0 (p<0.05; OR 10.5) genotypes significantly increased in group 2. Total analysis of the GSTM1, GSTT1 and NAT2 genes revealed significantly higher frequency of functionally impaired genotypes in group 2 as compared to group 1 (68.6% and 13.0% respectively, p<0.001,OR 14.6). These data suggest that the polymorphisms metabolisms genes is associated with different effect of hormone therapy in endometriosis patients.

P0921. Vascular ependymoma, a newly recognized ependymal tumor entity including clear cell ependymoma, is associated with chromosome 19 trisomy

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Monosomy 22 and gain of chromosome 7 are more frequent in spinal cord ependymomas while monosomy 9, gain of chromosome 1q, and loss of 6q and 13, are more frequently observed in intracranial ones. In the present study, we analyzed the genomic profile of monosomy 9 ependymal tumors in order to better understand their tumorigenesis and clinico-pathological characteristics. Using microsatellites on chromosome 9, we selected in a series of 147 ependymal tumors, 9 tumors with a monosomy, and one with loss of the p arm. Most of these tumors shared common clinical and histological features, reminiscent of a rare tumor, the clear cell ependymoma. Subsequently, we performed array-CGH on an enlarged series of tumors chosen on the basis of their histological appearance. We observed trisomy 19 to be the most frequent chromosomal alteration in this series. Architecturally, the tumors were characterized by compact aspect, presence of branched capillary network, and regularly dispersed cells. Some of them presented foci of clear cells.

In conclusion, our results allow us to describe a new sub-group amongst ependymal tumors, the vascular ependymoma to which clear cell ependymoma belongs. They are characterized by trisomy of chromosome 19 frequently associated with monosomy 9 and interstitial deletion of 13q. Such a finding is of importance for further research on specific pathways involved in ependymal tumor tumorigenesis. These data also underscores the heterogeneity within ependymal tumors and the need for further genetic dissection, a mandatory step to establish targeted treatment of ependymal tumors.

P0922. Implication of chromosome 18 in essential hypertension by sib-pair analysis and association studies: putative involvement of the *RKHD2* gene

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Essential Hypertension (EH) is a common risk factor for cardiovascular diseases, end stage renal disease, stroke and peripheral vascular diseases. We tested the implication of loci on chromosomes 9, 17 and 18 in the hypertensive phenotype by combining sib-pair linkage analysis and case-control association studies. The selection of these chromosomal regions was based on previous evidences from comparative genomics in rat models and from genome-wide linkage studies in humans. For the sib-pair analysis, a total of 27 microsatellites were genotyped in 58 pedigrees with hypertensive sibling-pairs from Spain. Linkage analysis showed significant excess allele sharing at the D18S474 marker on 18q21.1, as shown by maximum likelihood of allele sharing methods (LOD=3.24 p=0.00011) and non-parametric linkage calculations (NPL=3.32, p=0.00044). On the contrary, we did not obtain significant results with any of the markers analysed on chromosomes 9 and 17. We then focused on the *Ring Finger and KH Domain Containing* gene (*RKHD2*), located 6 Kb distal from D18S474 and putatively involved in ubiquitination. We performed a case-control association study based on linkage disequilibrium in 112 hypertensive patients and 156 controls. We analysed two *RKHD2* tagged SNPs covering the entire gene, rs1941958 and rs1893379, and observed a significant overrepresentation of the 1-2 *RKHD2* haplotype in the group of hypertensive patients in comparison to controls (2P=0.0004; OR=2.32). We also detected epistatic effects between the two *RKHD2* SNPs (2P=0.002; OR=3.5). Our data confirm the implication of chromosome 18 in EH and supports a contribution of *RKHD2* to the genetic susceptibility of this complex phenotype.

P0923. A novel recurrent mutation in ATP1A2 gene in a Portuguese family with familial hemiplegic migraine

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Familial hemiplegic migraine (FHM) is an autosomal dominant subtype of migraine with aura, with hemiparesis characterizing the attacks. Genetically, FHM families can be linked to 19p13 presenting mutations in the CACNA1A gene (FHM1), or linked to 1q23 with mutations in ATP1A2 gene (FHM2). Recently, a third gene for the disease was reported in 2p24, the SCN1A gene (FHM3). For FHM1 several recurrent mutations have been described and allow a more accurate genotype-phenotype correlation. For ATP1A2 however, recurrent mutations are described only once, and other mutations are present in single (small) families. Our aim was to investigate the involvement of ATP1A2 gene in a Portuguese family with pure FHM. Six multiallelic markers surrounding the ATP1A2 gene were genotyped and haplotype analysis was compatible with involvement of the ATP1A2 locus. Scanning the ATP1A2 gene for mutations was performed by direct sequencing of all exons and flanking regions, using genomic DNA of one patient of the family. Thus we identified the M731T mutation, previously identified in a Dutch pure FHM family, and confirmed cosegregation of the mutation with the disease phenotype in our family. Comparison of haplotypes of both the Dutch and the Portuguese family with the M731T mutation indicated that the mutation is recurrent rather than result of a common founder effect. This mutation is disease-causative in our family, also because recent functional and kinetic consequences on ATPase pump functioning were reported. Comparison of the clinical features in the two families revealed a clear association with pure FHM for the M731T mutation.

P0924. 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 usually diagnosed between ages 50-70 yrs. Up to 3% of IPF patients have a familial form of the disease (familial pulmonary fibrosis or FPF). In 2001, autosomal dominant mutations in the *Surfactant Protein C gene* (*SFPTC*) were shown to cause familial forms of lung disease, including FPF.
We have identified 14 Newfoundland (NL) FPF kindreds, from which 48 affected individuals and 268 unaffected family members have been recruited. We have also assembled a cohort of 24 "sporadic" PF patients. The mean age of diagnosis in the familial group was 59 years, compared with 63 years in the sporadic group (not statistically significant). However while no patient in the sporadic group was diagnosed prior to age 40, almost 20% of the familial group were diagnosed by this age, suggesting that FPF should be considered in any NL patient diagnosed with PF prior to 40. Newfoundland's minimum prevalence of FPF is 120 cases/million, which is over 100-fold greater than that of the United Kingdom, so that the NL population appears to be enriched for this Mendelian disorder.
SFPTC has been excluded in these 14 kindreds by immunohistochemistry and DNA sequencing. Two of the families are large, with 7 or more affected individuals, and are conducive for gene mapping using a traditional linkage analysis approach. Therefore, genome-wide scans using microsatellite markers have been performed. This analysis has identified several regions of interest which are being examined more thoroughly.

P0925. Limb girdle muscular dystrophy 2I in Spanish and Croatian population

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Limb Girdle Muscular Dystrophy type 2I (LGMD2I) is an autosomal recessive muscular dystrophy caused by mutations in the Fukutin Related Protein (*FKRP*). This gene is located at 19q13.3 and contains four exons. Immunohistochemical analysis is not possible to date because no specific antibodies have been isolated. Thus, mutational analysis is the method to obtain an accurate molecular diagnosis of the disease. The aim of this work is: 1) to diagnose the patients with clinical features suggesting LGMD2I, 2) to observe the frequency of this pathology in these two populations and, 3) to offer genetic counselling to the affected families.
We studied 60 Spanish and 10 Croatian patients presenting a LGMD2I phenotype and in whom we previously excluded a dystrophinopathy, LGMD2A, LGMD2C and LGMD2D. The molecular study was performed following two steps:
1) The analysis of the L276I mutation (reccurrent in North European population) by restriction enzyme analysis.
2) The sequencing of the unique coding exon 4.
We found four missense mutations in seven patients: E55Q, R143S, L276I and G373S, two of them were novel mutations: E55Q and G373S. Their pathogenia were demonstrate analysing 50 chromosomes from normal controls and 50 chromosomes from other myopathy patients.
The L276I mutation was identified in four patients (3 Spanish and 1 Croatian) and should be considered the more frequent *FKRP* mutation in the populations studied. The low number of *FKRP* mutations identified despite the patients were very well classified could indicate the low frequency of LGMD2I in these two populations.

P0926. Five years study of Fragile X Syndrome

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The Fragile X syndrome is the most common form of inherited mental retardation. It is caused by expansion of CGG triplet repeats in Fragile X Mental Retardation 1 (FMR1) gene. Normal individuals have <50 CGG repeats and permutation carriers have 50-200 repeats, but affected individuals whom are known as full mutation have over 200 repeats which are generally hypermethylated and this leads to transcriptional silencing of the encoded Fragile X Mental Retardation Protein (FMRP).
The objective of this study was to confirm the clinical observations with positive result using molecular genetics methods. In this regard we have examined a total of 268 unrelated mental retard individuals

from two centers, Genetics Research Center (GRC) and Kariminejad and Najmabadi Pathology and Genetics Center. These families had pedigree which were suggestive of X-Linked and were clinically suspected for Fragile X syndrome. In order to detect the CGG repeats in FMR1 gene, we used PCR and Southern blot analysis with non radioactive DIG labeled StB12.3 probe.
We identified 85 probands with Fragile X syndrome which represent 31.7% of tested probands (85/268). Prenatal testing was performed following a positive carrier test in 14 mothers; According to the results from 10 CVS (Chorionic Villus Sampling) and 4 amniocentesis, 3 out of 6 females and 2 out of 8 males had full mutation for CGG repeats.

P0927. CGG repeat transmission in an extended FRAXA Family from South India

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Fragile X syndrome is a common form of inherited mental retardation, affecting 1 in 4000 males and 1 in 8000 females. This was the first triplet repeat disorder discovered, with the CGG repeat polymorphism in the first exon of the *FMR1* gene. Since the discovery of the *FMR1* gene responsible for the fragile X syndrome, molecular diagnosis of fragile X is widely used, specially Southern blot analysis was found to be of great use in determining the mosaic pattern in the number of CGG repeats as well as methylation of CpG island. The mechanism leading to mosaicism of the CGG repeat is still elusive. We have screened 132 unrelated mental retardation cases, of which 7 cases were found to be positive for fragile X syndrome. An extended family of 5 generations has been identified, in this family total of 11 affected males, 3 mosaic females, 3 carrier females and a permutation carrier male have been identified using stb12.3 probe. Of the 11 affected males 3 were mosics with varying methalytion status. In the first patient we report a mosaic pattern of normal, premutation and full mutation, he has two sibs ,one male and one female,.who were found to be clinically normal. we have anlysed CGG repeat status in his sperms and in his sibs to see the transmission of CGG repeat status from mosaic male to his sibs.

P0928. Frequency of carriers for Friedreich ataxia in the Portuguese population

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Friedreich ataxia (FRDA) is a neurological disorder, caused by a large GAA repeat in intron 1 of the *FRDA* gene. FRDA is the most common early-onset recessive ataxia in Caucasian populations. Its prevalence was estimated to be around 1:25,000-1:50,000, while the estimated frequency of carriers is 1:60-1:120 in most Indo-European populations.
We followed an affected family for genetic counselling and cascade-testing, through which 3 expansion carriers were found in the general population (spouses). This prompted us to study the frequency of carriers in the Portuguese population, in anonymized Guthrie cards from IGM (Medical Genetics Institute, Porto; courtesy of Dr. Maximina Pinto).
We have thus analyzed 1059 blood spots (529 females, 530 males), uniformly distributed by the 20 districts of Portugal, according their population density. DNA was extracted quantified and tested for the *FRDA* $(GAA)_n$ expansion, by PCR, electrophoresis and Southern blotting.
A total of 2118 alleles were sized for their GAA repeat. Small normal alleles (<12 GAA) represented 94,2% and large normal alleles (12-33 GAAs) 5,1%. We found 10 expansion carriers (66 -1700 GAAs), with an aleatory geographic distribution, as well as 3 pre-mutation carriers (34 - 65 GAAs). Carrier frequency was estimated to be 1:106. This is in agreement with values mentioned in the literature for other European populations. This information will be important to foresee the needs for testing and do better risk estimates in counselling of families affected.

P0929. Toward the identification of the gene responsible for FTDU-17

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Ubiquitin-positive frontotemporal dementia (FTDU) is the most common pathological FTD subtype characterized by ubiquitin-positive, tau-negative inclusions in the dentate gyrus and in the superficial layers of the fronto-temporal cortex.
During our genetic-epidemiological study aimed to obtain a full ascertainment of FTD patients in the Netherlands we identified two large FTDU-17 families where we found linkage to chromosome 17q21. By fine mapping we were able to exclude the MAPT gene in which mutations have been previously found in several FTD families linked to 17 (FTDP-17) and significantly refine the linkage interval to approximately 3.4 cM. The reduced critical region is located in a gene-rich chromosomal area with 118 known genes. In our effort to identify the gene responsible for FTDU we have already excluded mutations in 30 functional positional candidate genes but a daunting mutation analysis effort would be required to investigate them all. Arguing that a common founder effect may take place for FTDU-17 as it did for the FTDP-17 MAPT-P301L mutation in the Netherlands we are using a linkage disequilibrium approach to further reduce the FTDU-17 critical region. We are currently genotyping 96 tag SNPs on seventy unrelated FTD familial cases to identify a shared ancestral chromosomal segment, where the responsible gene would be located. This approach will reduce the size of the critical region allowing us to focus on a smaller number of genes.

P0930. Genetic and clinical investigation of familial hematuria. Many patients develop progressive chronic renal failure from focal segmental glomerular sclerosis

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We investigated the genetic causes that led several of our patients to progressive renal failure and sometimes to end-stage renal disease with the characteristic histopathologic finding of focal segmental glomerular sclerosis (FSGS). We studied 90 patients and healthy relatives from 12 families who presented with autosomal dominant FSGS, as a primary or secondary finding in renal biopsies. We performed genetic linkage analysis on four chromosome regions with candidate genes that have been linked to the development of FSGS. Those are: 19q13 (ACTN4), 11q22 (TRPC6), 6p12 (CD2AP) responsible for primary FSGS, while gene locus 2q36 (COL4A3/COL4A4) has been linked to familial hematuria, thin basement membrane disease (TBMD) and secondary FSGS. Clinical investigation led to the finding of familial hematuria as the initial symptom as well as the identification of young subjects who had inherited the affected haplotype without proteinuria or renal failure. Haplotype analysis showed that 8 families are genetically linked to locus 2q36, 3 to locus 19q13 and one family to 11q22. For 2q36 the total LOD score is 7.7, clearly implicating either one of collagen genes COL4A3/COL4A4. Mutations in genes COL4A3/COL4A4 are responsible for three syndromes: Autosomal recessive Alport, autosomal dominant Alport Syndrome and autosomal dominant TBMD with progression to chronic renal failure and FSGS. These three syndromes present with phenotypes that are part of a continuous spectrum of symptoms not always permitting a clear diagnosis. Identification of the mutations will significantly facilitate differential diagnosis in cases of vague or complex clinical presentation, enabling timely and suitable therapy.

P0931. Mapping quantitative trait loci for expression of disease susceptibility genes

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Heritable differences in gene expression are considered to play an important role in susceptibility to complex traits, including many human diseases. Genetic influences on gene expression may be *trans*-acting as well as *cis*-acting, and are thus not limited to the gene locus itself. Recent studies have demonstrated that expression of a gene is a phenotype that is amenable to linkage analysis. These studies have also indicated that a large proportion of the heritable variance in gene expression is attributable to *trans*-acting polymorphism. Our studies are based on the principle that if *cis*-acting variation within a 'disease gene' influences susceptibility to disease through effects on that gene's expression, then genes containing *trans*-acting polymorphism with the same effect on the disease gene will also be susceptibility genes for the disorder. For complex disorders, the effect size of these loci will necessarily be greater on the intermediate expression phenotype than on the clinical phenotype, and thus should be more easily detected. As a method for mapping novel susceptibility loci, we have conducted linkage analysis of the expression of several susceptibility genes for neuropsychiatric / neurodegenerative disorders, using real-time PCR measures in large CEPH pedigrees. Analysis of the schizophrenia susceptibility gene *DTNBP1* will be presented.

P0932. Complex approach for the estimation of Renin-Angiotensin-Bradykinin sytem genes polymorphism contribution in arterial hypertension in children

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Renin-angiotensin-bradykinin system (RABS) is a regulator of arterial (blood) pressure and water-salt balance in human. A lot of genetic polymorphisms of this system are associated with cardiovascular diseases. However, proper interpretation of these multi-gene associations usually encounters substantial problems in evaluation of particular gene polymorphism contribution in pathogenesis of the disease. So, there is a clear cut necessity for the development of new approaches more objective evaluation of gene testing studies.
The "score" analysis amenable for the standard Mann-Whitney U test is applied. The polymorphisms of the REN (I9-83G>A), AGT (M235T), ACE (I/D), AGTR1 (1166A>C), AGTR2 (3123C>A), BKR2 (-58T>C и I/D) genes have been studied by PCR and PCR/biochip analysis in children with arterial hypertension (N=179) and in the relevant population group (N=158). The "score" analysis has been shown that polymorphisms of renin-angiotensin-bradykinin system genes plays essential role in development of arterial hypertension in the boys as well as in pathogenesis of stable form of arterial pressure in girls.

P0933. Toward the identification of the gene of the glomerulopathy with fibronectin deposits.

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Glomerulopathy with fibronectin deposits (GFND) is an autosomal dominant renal disease, which is characterized by glomerular fibrillary deposits that show strong immune reactivity to fibronectin. Patients with GFND develop albuminuria, microhematuria, arterial hypertension, renal tubular acidosis type IV, and ESRD disease in the second to sixth decade of life. Few clinical reports have been reported to date. The genetic cause of GFND is still unknown. A candidate gene approach strategy (uteroglobin gene and fibronectin gene) did not sort any result. A subsequent genome-wide linkage analysis revealed a critical region of 4.1 cM in 1q32. Mutation analysis of three candidate genes among 93 genes contained in the critical region failed to detect any pathogenetic mutation. We report here an accurate clinical and molecular characterization of a large family with GFND. Biological samples were obtained from 22 subjects: 7 alive patients, 14 healthy relatives and 1 deceased patient. Haplotype analysis allowed us to narrow the critical region. Mutational analysis of candidate genes is ongoing.

P0934. No evidence of del(GJB6-D13S1830) mutation in prelingually non-syndromic hearing impaired Croatians and Slovenians

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About 1 in 1000 children is affected by hearing loss at birth or in early childhood (prelingual deafness). At least 50% of cases are attributed to genetic factors. Among hereditary non-syndromic deafness, autosomal recessive forms predominate. The DFNB1 related disorder, caused by mutations in the GJB2 and GJB6 genes, accounts for 50% of autosomal recessive non-syndromic hearing loss. Approximately 98% of individuals with DFNB1 related disorder have two identifiable GJB2 mutations and 2% of them have one identifiable GJB2 mutation and one of two large deletions of GJB6. We examined 119 subjects with prelingual non-syndromic hearing impairment (63 Croatians and 56 Slovenians) by polymerase chain reaction, for the presence of the 35delG/GJB2 and the del(GJB6-D13S1830) mutations. The 35delG/GJB2 mutation was found in 21 Croatian subjects (33.3%) and in 28 Slovenian subjects (50%). In 11 of them the mutation was found in the heterozygous state and sequenceing revealed a second mutation in 10 of them (compound heterozygotes). The del(GJB6-D13S1830) mutation was not found in our group of patients. Our results contribute to the knowledge of geographic distribution and population genetics of the GJB2 and GJB6 mutations in the Europeans.

P0935. Glomuvenous malformations are caused by lack of glomulin, a specific marker of vascular smooth muscle cell differentiation

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Glomuvenous malformations (GVMs) are localized cutaneous vascular lesions, histologically characterized by the presence of mal-differentiated smooth muscle-like glomus cells around distended vein-like channels. GVMs are often hereditary and transmitted as an autosmal disorder. The gene was linked to chromosome 1p21-22 and named glomulin (glmn) (Boon et al, 1999). To date, 27 different inherited mutations have been identified. The majority of the changes cause the appearance of a premature stop codon and probably result in non-sense mediated mRNA decay (Brouillard et al, 2002 & 2005). Nevertheless, haploinsuffisance may not be sufficient to create a lesion, as a "second-hit" mutation was identified on the second allele in one lesion (Brouillard et al, 2002 & 2005).
By in situ hybridisation, we have shown that glomulin is specifically expressed in vascular smooth muscle cells (VSMCs) during murine development (McIntyre et al, 2004). We now show that glomulin expression begins in VSMCs after desmin, h-caldesmon and smooth muscle myosin heavy chain, but before the expression of smoothelin-b (McIntyre et al, submitted). We also demonstrate that glomus cells in GVMs do not express glomulin nor smoothelin-b, in contrast to venous malformations (McIntyre et al, submitted). This complete lack of glomulin supports the notion of GVMs being inherited in a paradominant fashion.
We conclude that glomus cells have been deviated in their differentiation process due to a complete lack of glomulin expression. (vikkula@bchm.ucl.ac.be)

P0936. Polymorphisms of the glutathione *S*-transferase *GSTM1, GSTT1* and *GSTP1* loci: possible protective role of *GSTP1* Val105Val genotype in embryogenesis.

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The aetiology of early pregnancy loss remains unclear, but it may be multifactorial with a possible genetic predisposition and involvement of environmental factors. Genetic polymorphisms in glutathione S-transferases (GSTs) resulting in altered detoxification may contribute to increased exposure of the conceptus to endo- or exogenous toxins and therefore play a role in the individual susceptibility to early pregnancy loss. The aim of this study was to investigate the selective role of *GSTM1, GSTT1* and *GSTP1* polymorphisms in the human embryo viability. We studied 171 embryos with normal karyotype that had been spontaneously aborted between the 6th and 12th week after conception and 135 adult controls for the *GSTM1* deletion (null), the *GSTT1* deletion (null) and the *GSTP1* Ile105Val functional polymorphisms. *GSTM1* and *GSTT1* null genotypes were determined in 84 (49%) and 42 (25%) spontaneous abortions, and 74 (55%) and 32 (24%) controls, respectively. *GSTM1* and *GSTT1* genotype distributions did not differ significantly between these two groups. Regarding *GSTP1* genotypes, the proportion of Val105 homozygotes was significantly lower in the spontaneous abortion group than in the control individuals (5% versus 13%, *P*=0.02). In conclusion, the *GSTP1* Val105Val genotype may be protective in the early human development.

P0937. Analysis of the *HMOX1* gene in South African patients with variegate porphyria

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Variegate porphyria (VP) demonstrates substantial variability in clinical presentation. More than 90% of South African VP patients present with the protoporphyrinogen oxidase gene (*PPOX*) R59W mutation due to a founder gene effect. However, phenotypic variations are observed between these individuals, suggesting the involvement of yet unidentified modifier genes. A potential candidate modifier gene is the haem-oxygenase 1 gene (*HMOX1*) that is involved in cleavage of the haem ring at the alpha methene bridge to form biliverdin. In this study we investigated the *HMOX1* gene as a possible modifier locus for VP in the South African population. The study population consisted of 25 well-characterised R59W heterozygous VP patients, including patients with skin symptoms (11), acute attacks (2), acute attacks in combination with skin lesions (5) and asymptomatic patients (7). PCR amplification was performed on the promoter and coding region of the *HMOX1* gene followed by heteroduplex single-strand conformation polymorphism (HEX-SSCP) analysis. Subsequent DNA seqeuncing revealed several variants in the promoter region [$(GT)_{13}$; $(GT)_{21}$; $(GT)_{23}$; $(GT)_{27}$; $(GT)_{15}$at$(GT)_{14}$; $(GT)_{13}$at$(GT)_{20}$], intron 2 (IVS2-19C→T and IVS2-31T→C), exon 4 (R237G) and intron 5 (IVS5+51delTGGCTGTC TGACT). The variants IVS2-19C→T (2 of 7) and R237G (1 of 7) were identified only in the asymptomatic group, comprising 43% (3 of 7) of this group. Variants identified exclusively in the patient group were observed in 12% of this group. This study demonstrates the potential involvement of *HMOX1* variants as a protective mechanism against the development of symptoms associated with VP in R59W mutation individuals and warrants further studies.

P0938. Linkage Analysis of the ATxTy genotype on Haemoglobin F Malta I in the Maltese population

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Introduction: HbFMaltaI or [α2γ2 2117(G19) His->Arg] is a haematologically and clinically benign Gγ globin variant found in 1.8% of the Maltese newborn. It is linked to a stable abnormal haemoglobin, HbValletta or [α2β2 287(f3) Thr->Pro]. The ATxTy motif 5' to the β globin gene was investigated by genetic linkage analysis. Methods: A total of 112 samples (224 chromosomes) that included 61 HbFMaltaI and 51 normal Hb were analyzed. DNA sequencing was carried out at position -530bp comprising silencer II ATxTy polymorphism and -300bp comprising silencer I 69969 C->T SNP. The HbFMaltaI were further typed for the XmnI genotype. Results: 69969C/T was in HWE for both HbFMaltaI and normal controls. (AT)xTy was in HWE for normal controls but not for the HbFMaltaI (χ^2=17.34; p<0.005). Two HbFMaltaI homozygotes carried the homozygous $(AT)_9(T)_5$ allele. Genetic data analyzed by Linkage Disequilibrium Analyzer (LDA) and PHASE v2.1.1 software strongly suggests linkage disequilibrium (LD) of the $(AT)_9(T)_5$ allele with HbValletta and HbFMaltaI (χ^2=93.30; p<0.001; D'=1.00; LR: p<0.001) in the Maltese population. A statistically significant association between (AT)xTy genotypes and the XmnI site in HbFMaltaI (χ^2=7.6; p=0.023) was also observed. Discussion: The $(AT)_9(T)_5$ allele as shown by the HbFMaltaI homozygote samples, occurs on the same haplotype. All HbFMaltaI heterozygotes carried at least one of this allele type. The $(AT)_9(T)_5$ is in tight linkage disequilibrium with HbValletta and HbFMaltaI. HbFMaltaI may therefore serve as a model to understand better, both at the gene and protein level of interactions that occur *in vivo*.

Estimated Haplotypes by PHASE software v2.1.1

Haplotype	Haemoglobin	69758C/T	ATx	Ty	69969C/T	Allele count
1	HbF	t	7	7	t	55
2	HbF	t	7	7	**c**	25
3	HbF	c	7	7	t	33
4	HbF	c	9	5	t	37
5	HbF	c	9	5	c	2
6	HbF	c	11	3	t	9
7	HbFMaltaI	c	9	5	t	63
					Total	**224**

P0939. Genetic and functional mechanisms of ABCA12 associated Harlequin Ichthyosis

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Harlequin ichthyosis (HI) is the most severe recessive congenital ichthyosis. Due to impaired barrier function of the skin neonates have often died within 2 days of birth. Using SNP chip technology and subsequent sequencing our previous studies detected that mutations in the gene encoding ABCA12 [(ATP)-binding cassette transporter] underlie the skin disease HI in 11 out of the 12 patients studied. In this study we have sequenced the ABCA12 gene of a further 13 patients, the results show all of them contain either nonsense substitution or frameshift mutations. Non-denaturing HPLC analysis and Agilent DNA oligo arrays showed the presence of a heterozygous whole exon deletion in a patient with no mutations previously located with standard sequencing. These mutation data establish *ABCA12* as the major HI gene.

Immunofluorescence demonstrated completely absent ABCA12 protein expression in HI skin (n=2) with expression throughout normal, control epidermis, most strongly in the stratum granulosum. Furthermore markers of late epidermal differentiation, such as transglutaminase 1 and ZO-1, had a highly abnormal distribution in HI skin being expressed in the lower layers of the epidermis including the basal cell layer. In summary mutations have been found in all HI patients studied to date and exon copy number experiments should be considered when screening for HI. Immunostaining has proven that this protein is completely absent in HI skin and that the normal pattern of late epidermal differentiation is severely impaired. These data suggest an important role for ABCA12 in early epidermal differentiation in addition to effective barrier function.

P0940. Genetic studies of the Iranian deaf population

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During last six years, total of 1395 families with hearing loss have been referred to our center for genetic testing. Thirty eight families had syndromic hearing loss. Majority of families had autosomal recessive non syndromic hearing loss (ARNSHL) pattern of inheritance. In 97 probands, no other affected relative could be identified - we classified these as simplex cases. *GJB2* mutation screening was complete in 1246 patients with presumed ARNSHL; initially we tested for 35delG mutation. Persons either negative or heterozygous for this mutation were analyzed by denaturing high performance liquid chromatography and direct sequencing. We found *GJB2*-related hearing loss in 210 of 1246 (15.6%) ARNSHL cases. Identified deafness-causing allele variants included 20 mutations which 507insAACG, 329delA, 363delC and Q80L are the novel mutations and they have not been reported in other populations. The objective of this study is to identify the gene(s) involve in ARNSHL in Iranian Populations. As the first step the 14 known loci of ARNSHL with three and more affected individuals are being investigated by homozygosity mapping using STRs Markers. Whole Genome Screening using SNP typing has been performed for the families which can not be localized to the known loci. So far over 6 years, we have been able to collect over 722 families with two or more affected deaf and our linkage result have identified 3 new Loci which have not been mapped previously.

P0941. Mutations in *GJB2*, *TMC1*, *TMPRSS3* and *MYO15A* cause autosomal recessive nonsyndromic hearing loss in Turkish patients

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Autosomal recessive nonsyndromic hearing loss (ARNSHL) is the most common form of hereditary hearing impairment. Although high genetic heterogeneity, mutations in the *GJB2* (*DFNB1*) are a remarkably frequent cause of ARNSHL in all populations studied so far.

Ninety unrelated familial patients with ARNSHL from the northeast of Turkey were evaluated. Screening for mutations in the *GJB2* gene revealed five already described mutations, p.G12*fs*X13 (35delG), p.W24X, p.R104*fs*X109 (310del14), p.E120del and p.R184P. In addition, two novel mutations, p.Q80K and p.P173S, were identified. In total, these mutations explain the ARNSHL in 29 (32.2%) of the patients. 35delG was the most common mutation, accounting for 76% of all mutant *GJB2* alleles.

A number of families with a single or without a *GJB2* mutation and suitable for homozygosity mapping were selected and analysed. Homozygosity was detected in four families for *TMC1 (DFNB7/11)*, in three families for *TMPRSS3 (DFNB8/10)* and in one family for *MYO15A* (DFNB3). Subsequent sequencing of the coding region and exon-intron boundaries of these genes revealed four novel *TMC1* mutations, p.Y259C, p.P274L, p.R445H, R362*fs*X367, in 4 (4.4%) families. In *TMPRSS3*, one known mutation, p.P404L, and two novel mutations, p.R216L and p.Q398X, were found in 3 (3.3%) families. In *MYO15A,* one novel mutation, p.G1831V, was identified in 1 family.

Our results indicate that besides mutations in *GJB2*, mutations in *TMC1* and *TMPRSS3* contribute remarkably to ARNSHL, especially considering the high level of genetic heterogeneity of ARNSHL.

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P0942. DFNB1 mutations are not the most frequent cause of hearing loss among Iranian population

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Background: Congenital profound deafness has a known genetic origin in more than 50% of all cases. The majority of the non syndromic hearing loss shows an autosomal recessive inheritance pattern. DFNB1 is characterized by congenital, non-progressive mild-to-profound sensorineural hearing impairment. This locus contains GJB2 and GJB6 genes that encode connexin 26 and connexin 30, respectively. Mutations in the GJB2 gene account for more than 50% of the recessive non syndromic deafness.

Materials and Methods: In this study, we investigated 50 consanguineous families with at least two affected individuals that already had been tested negative for mutations in GJB2 and GJB6 genes for linkage to DFNB1 locus.

Results: In order to find the linkage, genetic linkage analysis with three STR markers (D13S787, D13S633, and D13S292) was performed and none of the 50 families was linked to DFNB1.

Conclusion: This data suggest that it is unlikely that other genes in this region are involved in autosomal recessive non-syndromic hearing loss (ARNSHL) in Iranian population.

P0943. The FVIII gene polymorphisms in Moldovan patients with hemophilia A

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Hemophilia A is a common X-linked bleeding disorder affecting approximately 1 in 5000-10000 males. It is caused by various mutations in the FVIII (FVIII) gene, leading to deficiency of functional coagulation. We report the results of polymorphism analysis on a series of Moldovan patients with hemophilia A to evaluate its value for carrier detection.

The study was performed in 42 unrelated male patients with hemophilia A. Relatives from their families were also studied for carrier detection. Total genomic DNA was extracted from peripheral blood according to standard procedures. A single nucleotide polymorphism in the FVIII gene HindIII/intron19 and an extragenic St14 (DXS52) VNTR were studied by PCR-based methods. Also these markers were analyzed in control group (64 unrelated X-chromosomes).

The presence of the restriction site HindIII+ was observed in 40.5% of hemophilia A patients. The frequency was similar to that observed in controls (35,9%). Determination of the length of the St14 VNTR led to observation of twelve alleles ranged from 670bp to 2900bp. The 1690bp-allele was the most frequent allele with 42% and 35% frequency in patients and in control group, respectively. The expected heterozygozity rate was 0.787. Of 42 families with hemophilia A 36 (86%) were informative for these two polymorphisms. Twelve female relatives (except patients' mothers) from 11 hemophilia A families were analyzed for carries status. In 7 of them the carrier status was rejected.

Thus, the results indicate that these polymorphisms are suitable for the carrier detection and prenatal diagnosis in Moldova.

P0944. The second most prevalent locus (DFNB4) in the Iranian patients with hearing loss

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Background: The mutation in SLC26A4 gene in DFNB4 locus is responsible for syndromic(Pendred syndrome) and non-syndromic hereditary hearing loss(HHL). In many population the mutation in this gene have been reported as the second cause of HHL. The objective of our study was to investigate the prevalence of SLC26A4 mutations in our HHL consanguineous families.

Materials and methods: After complete clinical examination the consent form was taken from each family. we included 77 families with more than two affected individuals, who have been referred to GRC (Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran). All families had previously tested negative to DFNB1 locus, were candidate for homozygosity mapping using STRs for DFNB4 locus. Families localize to this region were subjected complete DNA sequencing.

Results: Ten out of seventy seven families were mapped to DFNB4. Sequence analysis of five linked families revealed six mutations (T420I, 1197delT, G334Y, R409H, R79X, T721M, R79X) that T420I , G334V and R79X were novel mutations. Mutations detection for the other families is performing.

Conclusion: We have been all to localize total 10 families (13%) to DFNB4 locus. Five families had thyroid dysfunction(pendred syndrome) and in two families we couldn't find any symptoms of thyroid impairment, for the rest of families thyroid function testing being investigated. Our result demonstrate that SLC26A4 gene mutation is the second most prevalent cause of HHL in Iran. This result is in accordance with most of the other reports from other countries which have been studied.

P0945. Genes PMP22 duplication screening in patients with hereditary motor and sensory neuropathy from Bashkortostan

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Hereditary motor and sensory neuropathy (HMSN) is genetically heterogeneous disorder of peripheral nervous system, characterized by progressive weakness, atrophy of the peroneal muscles and distal muscles of arms. The frequency of the disease varies from 10 to 40 cases per 100000 in different populations (Skre, 1974). The HMSN frequency in Bashkortostan Republic is 10,3:100000. The most frequent cause of the disease is 1,5 Mb duplication in chromosome 17p11.2-12, comprising peripheral myelin protein (PMP22). The mutation frequency varies in different populations.

The examined group of patients consisted of 136 HMSN patients from 96 families, living in Bashkortostan. 60 patients analyzed (62,5%) were clinically diagnosed as HMSN type I, 9 patients (9,38%) - HMSN type II, 1 patient (1%) - HMSN type IV and 1 patients (1%) - HMSN type V. HMSN type confirmation was required in 25 families. Gene PMP22 duplication analysis was performed using PCR-analysis of micro- and minisatellite DNA-loci in all patients without differentiation diagnoses for HMSN types. Gene PMP22 duplication was revealed in 38 patients from 30 unrelated families. The duplication frequency in unrelated patients was 31,25% for all types of HMSN and 50,85% - for HMSN type I , that appeared to be lower than in Western Europe countries, where the duplication frequency is 75-80% (Latour et al., 2001). In one examined family PMP22 duplication and HMSN, caused by this mutation, was found only in one family member, so, the detected mutation appeared to be mutation de novo.

P0946. Polymorphic sequence variations in Hereditary Hemorrhagic Telangiectasia genes

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Hereditary hemorrhagic telangiectasia (HHT) is a multisystemic vascular dysplasia characterized by direct arterio-venous connections. The prevalence of this autosomal dominant disorder is estimated to be 1 in 10,000. Although there is marked age-dependent and variable clinical expression, the most frequent findings include epistaxis, telangiectases, and arteriovenous malformations (AVM).

HHT is genetically heterogeneous with two known genetic forms. HHT1 and HHT2 are caused by mutations in the endoglin (*ENG*) and activin A receptor type II-like 1 (*ACVRL1*) genes, respectively. More than 90% of these mutations are novel and most of them are missense. Hence, differentiating missense mutations from polymorphisms is a major problem. In order to provide a reference, we have sequenced the coding regions and exon-intron boundaries of *ENG* and *ACVRL1* genes in 100 patients with HHT and 100 control individuals. We compared the frequency of the polymorphisms in HHT patients with controls.

We found 11 different sequence variants in the *ACVRL1* and 18 variants in the *ENG* gene. There is no difference in the frequency of the nucleotide changes between the patient and control groups. Thus none of these sequence variants suggest association with disease. Furthermore, knowing the polymorphisms in these genes will be useful in counseling the patients who have novel missense mutations.

P0947. Mutations of the *RET* gene in isolated and syndromic Hirschsprung disease disclose major and modifier alleles at a single locus

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Hirschsprung disease (HSCR, MIM 164761) stands as a model in the study of diseases with a complex mode of inheritance. A multiplicative oligogenic model with 3 loci has been proposed with *RET* proto-oncogene being the key player. Indeed, almost all HSCR patients harbor either a heterozygous mutation of the coding sequence or, more frequently, a hypomorphic allele located in a conserved non gene sequence in intron 1. In roughly 30% of the cases however, HSCR is associated to other malformations. Hitherto, the disease causing gene is known in 4 syndromic HSCR forms with mendelien inheritance among which CCHS (MIM209880, PHOX2B mutation) and Mowat-Wilson (MIM235730, *ZFHX1B* mutation). The penetrance of the HSCR phenotype is estimated about 20% for *PHOX2B* gene mutation and 60% for *ZFHX1B* mutation. To test whether *RET* could be regarded as a modifier gene for the enteric phenotype in these 2 syndromes, we genotyped the *RET* locus in patients for which the disease-causing mutation had been identified previously for CCHS (N=143) or MWS (N=30). Splitting patients into 2 groups (with or without HSCR) for each syndrome showed a statistically significant over representation of the RET hypomorphic allele in CCHS patients only (χ^2=11.52; p<0.001). Therefore *RET* acts as a modifier gene for the enteric phenotype to occur in patients with a *PHOX2B* gene mutation. These data illustrate the concept of a developmental gene being either a major disease-causing gene or a modifier gene. Finally, we also suggest the possibility of *RET* dependent and *RET* independent HSCR cases.

P0948. HLA match test by Real time PCR in buccal cheek swabs increase the feasibility for reaching higher number of donors.

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We have previously described a rapid HLA match test procedure that uses real time fluorescent PCR and melting curve analysis for common HLA-A, HLA-B and HLA-DRB1 alleles in DNA obtained from blood or from buccal cheek swabs. We have now applied the

protocols to screen 48 individuals among relatives of 5 allogeneic transplant recipients. Buccal cheek swabs have been used by the donors at rural areas without the help of a health professional and sent at room temperature by courier mails to our laboratory. HLA-A, HLA-B and HLA-DRB1 alleles have been tested by Real Time PCR using hybridization probes followed by melting curve analysis. The samples have been studied in parallel by commercial SSP PCR kits. The total turnaround time for HLA-A, HLA-B and DRB1 match test was 3 hours. Compared to the high costs, sample requirements and long turnover times of the HLA SSP typing tests, real time PCR dramatically reduced the costs while minimizing the required DNA amount thus enabling the use of buccal swabs for HLA match tests. Buccal swabs were easily used and sent to our laboratory by family members living at rural areas. This has expanded the availability of potential donors. Analysis of buccal cheek swabs by real time PCR using hybridization probes increase the feasibility for accessing to a higher number of HLA compatible donors.

P0949. Unexpectedly low prevalence of HLA-B27 in Romanian ankylosing spondylitis patients

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The association between ankylosing spondylitis (AS) and HLA-B27 locus is documented for some 30 years. However, the prevalence of HLA-B27 in different ethnic populations is not the same, both for the general population and ankylosing spondylitis patients. In this study we tested 40 AS patients and 50 healthy individuals from the Romanian population for the presence/absence of the HLA-B27. The testing was conducted by duplex PCR, with one primer pair amplifying a 135 bp fragment from the exon 3 of the B27 gene (in positive individuals), while another primer pair amplified a 357 bp fragment from the exon 4 of CD62E gene and functioned as an internal control of amplification. All the samples were amplified at least three different times, and consistent results were obtained in all cases. The prevalence of HLA-B27 in AS patients was 77.5% (31/40) and in healthy control population 14% (7/50). A chi-squared test was conducted (alpha=0.05) with expected values taken from the literature, respectively 90% for the prevalence of HLA-B27 in AS patients, and 8% for the prevalence of HLA-B27 in Caucasoid population. The test showed no significant difference for the prevalence of the investigated locus in the general population, but significant deviation from the expected values was found for the AS patients. This study revealed an unexpectedly low prevalence of HLA-B27 in AS patients from Romania.

P0950. Validation of the reshaped Shared Epitope HLA-DRB1 Classification in Rheumatoid Arthritis

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Objective. We proposed a classification of HLA-DRB1 alleles, reshaping the shared epitope hypothesis in rheumatoid arthritis (RA): RA was associated with the RAA shared epitope sequence (72-74 positions) and the association was modulated by the amino acids at positions 70 and 71, resulting in 6 genotypes with different RA risks. Here, we tested this classification for validation in an independent sample.

Methods. A new sample of 100 French Caucasian families with one RA patient and both parents was genotyped for the HLA-DRB1 gene. The alleles were grouped as previously: S_1 alleles for the sequences A-RAA or E-RAA, S_2 for Q or D-K-RAA, S_{3D} for D-R-RAA, S_{3P} for Q or R-R-RAA, and X alleles for no RAA sequence. Over or undertransmission of alleles was investigated. Genotypes odds ratio (OR) calculation was performed through a conditional logistic regression, and homogeneity of these OR with those of the 100 first trio families previously published was tested.

Results. The S_2 and S_{3P} alleles were significantly overtransmitted. The undertransmitted S_1, S_{3D} and X alleles were grouped as L alleles, as previoulsy. Under this 3 alleles classification, the risk hierarchy of the 6 resulting genotypes was, by decreasing OR: S_2/S_{3P}, S_2/S_2, S_{3P}/S_{3P}, S_2/L and S_{3P}/L, the reference genotype being L/L. Those results validated the proposed classification. Following an homogeneity test, we pooled the two samples and improved estimates from the highest (S_2/S_{3P}) to the lowest (S_{3P}/L) risk genotype.

Conclusion. Using an independent sample, we validated the classification previously described and provided estimates for the resulting genotypes.

P0951. HPFH and δβ-thalassemia in Iranian patients with β-thalassemia

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Backgrounds: Hereditary Persistence of Fetal Hemoglobin (HPFH) and δβ-thalassemia are heterogeneous disorders, characterized by increased level of fetal hemoglobin (HbF) in adult life. 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 HPFH and δβ-thalassemia. Here, we studied the most eight common deletions involved in HPFH and δβ-thalassemia in Iranian patients.

Materials and Methods: We included 17 patients who have referred to our private genetic center since last 3 years with elevated levels of HbF, and low MCV. After obtaining the informed consent, DNA was extracted from whole blood by salting-out method. Detection of 8 deletions including HPFH-1, HPFH-3, Spanish, Sicilian, Chinese, Asian-Indian inversion-deletion $^{G}\gamma(^{A}\gamma\ \delta\beta)^{0}$, and the Turkish form of inversion-deletion $(\delta\beta)^{0}$ thalassemia, Turkish (Inv-Del) was based on PCR method described previously by Craig et al.

Results: We found Sicillian, Hb lepore and Asian-Indian inversion-deletion $^{G}\gamma(^{A}\gamma\ \delta\beta)^{0}$ deletions causing δβ-thalassemia in 9(53%), 3(17.6%) and 4(23.5%) of patients, respectively. We could not find none of eight deletions in one of the patients.

Conclusion: Taken together, this is the first study of deletions involved in HPFH and δβ-thalassemia in Iranian patients with β-thalassemia that highlight the heterogeneity of the genetic background of Iranian population and importance of screening this deletions in prenatal diagnosis.

P0952. Familial presentation of Hereditary spastic paraplegia

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Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of inherited disorders characterized by insidiously progressive, often severe, lower extremity weakness and spasticity. Symptom severity, age of onset and rate of disease progression may vary widely among patients. Estimated prevalence is 5 in 1000000.

The most common pattern of HSP's inheritance is autosomal dominant.

Twenty-one loci have been linked to various forms of HSP; 10 loci have been mapped for autosomal dominant forms of HSP, seven loci for autosomal recessive forms and three loci for X-linked forms. Six genes associated with HSP have been identified: *SPG3A* (atlastin), *SPG4* (spastin), *SPG7* (paraplegin), *SPG20* (spartin), *L1CAM* (L1 cell adhesion molecule) and *PLP* (proteolipid protein).

SPG4 gene is the most common gene associated with HSP and we cheked it in our patients.

37 years old woman with foot paresthesia and paraplegia referred to us .Her illness began when she was 21 y. Both feet are affected but the right one is in better conditions. She suffers from back pain while walking too. Examination revealed increased muscle tone and proximal motor deficit in the lower limbs. Hyperreflexia in lower and

upper limbs, and Babanski signs are seen distal atrophy of food and prominent Lordosis and slight impaired vibration sense in ankles are seen.
Mother was normal, father suffered from spinal canal stenosis (first cousins). Her older brother and 22 years old son has similar complain. One of cousins has unknown paraplegia.
Neuroimaging,Electrophysiologic and genetics molecular diagnosis were done.

P0953. Association of obesity in psychiatric patients using antipsychotics with a HTR2C SNP genotype in the 3'UTR region is stronger than with promoter polymorphism genotypes

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The use of antipsychotic drugs is associated with obesity, and therefore an increased risk of cardiovascular mortality. As association of obesity in an unaffected and untreated Asian population with genotypes of SNPs within the promoter region of the HTR2C gene coding for the serotonine-2C receptor has been reported previously, but subsequent reports on the (lack of) association between antipsychotic-induced obesity and HTR2C genotype are conflicting. In this cross-sectional study the association of obesity (body mass index over 30 kg/m^2) in 127 psychiatric patients using antipsychotics with the HTR2C promoter region polymorphisms rs3813929:C>T (-759 C/T), rs518147: G>C (-697 G/C), rs3813929:C>T (-759 C/T), rs3813928:G>A (-997 G/A), rs1414334:C>G and HTR2C: c.1-142948(GT)$_n$), and a SNP (rs1414334:C>G) in HTR2C intron 5 close to the 3' UTR was evaluated. Association with obesity (evaluated with logistic regression and expressed as adjusted odds ratios (OR) with 95% confidence intervals (95%CI)) was observed in carriers of the variant alleles rs1414334: C (OR 2.80 (95%CI:1.03-7.62)) and HTR2C:c.1-142948(GT)$_n$:Z-6 (OR 2.30 (95%CI:0.92-5.78)). The haplotype carrying the variant alleles HTR2C:c.1-142948(GT)$_n$:Z-6, the wild type allele rs3813929:C, the variant allele rs518147:C, and the variant allele rs1414334:C was associated with an increased risk of obesity (OR 3.71 (95%CI:1.24-11.12)). In conclusion, some HTR2C polymorphisms are associated with obesity in patients taking antipsychotics, and the association appears to be stronger with a particular haplotype including the intronic SNP. Possibly, HTR2C promoter activity does not entirely determine this association, but it may well be that a more downstream alteration affecting HTR2C stability, expression, or even function is equally or more important.

P0954. Genome wide linkage analysis of a novel hereditary progressive hyperpigmentation disorder

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Familial progressive hypermelanosis is a new variant of a hereditary pigmentation disorders without associated symptoms. This phenotype has so far been observed only in five families, all living in close proximity in a small town in south-east of Germany (Zanardo et al., 2004). The phenotype, with an autosomal dominant inheritance with reduced penetrance, consists of progressive diffuse, partly blotchy hyperpigmentation, multiple café-au-lait spots, intermingled with scattered hypopigmented appearing maculae, and lentigenes. Histological and ultrastructural section from the hyperpigmented spots display strong basal hyperpigmentation of the epidermis with numerous melanophages containing large amounts of pigment. In contrast, the hypopigmented appearing macula show a slight basal hyperpigmentation of epidermis, but virtually no melanophages in the upper dermis.
The restricted area of occurrence of the disease and the extreme rarity of the phenotype suggest a common origin and thus a founder effect for this genetic defect. Based on this hypothesis, we performed a genome-wide linkage analysis in the five families using the GeneChip Human Mapping 10K Array SNP genotyping. The results demonstrate the presence of significant linkage peaks with maximal LOD of 2.28 assuming the smallest genetic distance between the five families, on the long arm of chromosome 12. All affected individuals shared the same haplotype in this locus. This finding suggests that a gene involved in melanin distribution is located on chromosome 12. (http://www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be)

P0955. An audit of a diagnostic service for Hypertrophic Cardiomyopathy and Dilated Cardiomyopathy.

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Since April 2004 the Oxford Genetics Knowledge Park, UK, has funded a molecular genetic service for Hypertrophic Cardiomyopathy (HCM) and Dilated Cardiomyopathy (DCM). Mutation screening by dHPLC and sequencing is available for the *MYH7, MYBPC3*, and *TNNT2* genes. To date, we have identified pathogenic variants in 38/76 HCM and 5/13 DCM patients. Mutation testing has been undertaken in 78 relatives, of whom 61% were found to have the familial mutation. An audit of the analysis performed and the mutations and polymorphisms identified will be presented. Implementation of new strategies to increase detection rate will be considered as will the need for high throughput technologies for the delivery of an effective cardiomyopathy service.

P0956. *SLC22A12* mutations of renal hypouricemia in Japanese

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Renal hypouricemia is an inherited and heterogeneous disorder characterized by increased urate clearance. We established that urate was reabsorbed via URAT1 on the apical membrane in the proximal tubules, and that mutations in SLC22A12 encoding URAT1 cause renal hypouricemia. In 2004, we reported the characteristics including serum urate levels, urate clearances and mutations in the 32 unrelated patients with idiopathic renal hypouricemia (J Am Soc Nephrol 15, 164, 2004). In this conference, we elucidate SLC22A12 mutations of 86 patients with idiopathic renal hypouricemia in 71 families.
Seventy-three patients either came to Jikei University Hospital or their doctors consulted with the hospital between January 1, 1998 and December 31, 2005. Thirteen patients came to Tottori University Hospital between January 1, 1998 and December 31, 2005.
Seventy-seven patients in 64 families had SLC22A12 mutations, 33 homozygotes, 23 compound heterozygotes, and 21 heterozygotes. We identified fourteen mutations. Nonsense mutation G774A dominated SLC22A12 mutations (82.7 % of 133 affected SLC22A12 alleles). G269A, G412A, Del1639-1643 and IVS2+1G→A were also identified in 12, 3, 3 and 3 of the alleles, respectively.
Our findings indicate that SLC22A12 is responsible for most renal hypouricemia and high frequency of G774A mutation should result in a number of the patients with renal hypouricemia in Japanese, comparing to the number of patients with renal hypouricemia in other countries.

P0957. Photosensitivity as an endophenotype to dissect common idiopathic generalized epilepsies: follow-up studies of the susceptibility 7q32 and 16p13 regions

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Common idiopathic generalized epilepsies (IGEs) occur frequently in the general population, representing a significant impact on human health. Progress towards understanding their genetic basis has been slow because of the complex inheritance patterns and genetic heterogeneity within phenotype definitions. Mapping susceptibility genes for clearly identifiable neurophysiological endophenotypes, such as variation in abnormal EEG responses to intermittent photic stimulation, also called photosensitivity or photoparoxysmal response

(PPR), may provide the means to dissect the genetic basis of IGEs. Recently, we mapped two susceptibility loci for PPR at 7q32 and 16p13 in 16 PPR-multiplex families with prominent myoclonic epilepsy background (MS-related PPR). Here we present the follow-up studies performed on these regions. The inheritance model for these two loci was explored, showing that the 16p13 locus may have a recessive inheritance. A two-locus linkage analysis was also performed to test for different interaction models and higher evidence for linkage under a multiplicative model (P= 0.001) was found, suggesting that both loci are necessary, but each alone is not sufficient, to predispose to MS-related PPR. Finally, sequence analysis of four plausible functional genes localized in the two loci regions, three neurotransmitter G-protein-coupled receptors (SSTR5, CHRM2 and GRM8) and one voltage-gated ion channel (CACNA1H), was performed in two patients from each family and in Dutch controls. Novel variants not reported in the NCBI dbSNP and several known variants were found and are currently being evaluated for familial segregation and putative biological meaning. The identification of susceptibility genes for PPR would advance our understanding of epileptogenesis.

P0958. Different HLA class II subtypes seems to be associated with immunoserological and clinical features of adult-onset idiopathic inflammatory myositis in Hungarian patients: results of a preliminary study

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Objectives Idiopathic inflammatory myopathies (IIM) are a heterogenous group of rare diseases characterized by muscular weakness. The autoimmune process may be induced by environmental factors in genetically susceptible individuals. Our aim is to investigate HLA class II associations with myositis-specific and -associated autoantibodies and clinical features in IIMs.

Patients and Methods DNA and serum samples were obtained from 33 polymyositis, dermatomyositis or overlap myositis patients, who were followed-up by a single center. Subjects were genotyped for HLA-DRB1, DQA1 and DQB1 by polymerase chain reaction with sequence specific primers technique. The presence of myositis-specific autoantibodies (anti-tRNA synthetases: Jo-1, PL-7, PL-12; anti-Mi-2) and myositis-associated autoantibodies (anti-PM-Scl and anti-Ku) were also examined.

Results Twelve patients were positive for MSAs or MAAs. We found 3 patients with anti-Jo-1, 3 with anti-PL-7, while none of them were positive for anti-PL-12. Anti-Mi-2 were detected in 3, anti-PM-Scl in one, and anti-Ku in 4 cases, respectively. All of the anti-Jo-1 positive patients carried the DRB1*03, DQA1*05 and DQB1*02 alleles and clinical features were typical to anti-synthethase syndrome. The HLA class II subtypes and clinical features differed among anti-PL-7 positive patients. The anti-Mi-2 were found in 2 dermatomyositis patients and in a polymyositis patient. One of the anti-Mi-2 positive DM patients carried DB1*07, DQA1*02 and DQB1*02 alleles.

Conclusion Haplotype associations may influence immunoserological status as well as phenotypic features common to the major forms of IIMs. Our further goal to examine possible associations among immunogenetic, immunoserological and clinical features and find prognostic factors which may predict therapeutic responses.

P0959. TNF-α and IL-10 genetic polymoriphisms influence the natural history and the response to antiviral therapy in HCV related chronic hepatitis

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Chronic hepatitis C (CH-C) develops in 33-84% of patients with acute hepatitis . About one-third of patients develop liver cirrhosis within 15-25 years with an annual risk of hepatocellular carcinoma in 1-4%. HCV positive patients with persistent normal ALT (PNAL) represent the 20-30% of the HCV population. Here, we verify the potential role of cytokines in inflammatory damage of CHC and in IFN therapy resistance. So, polymorphisms in IL-10 and TNF-α (-1082 G/A and -308 G/A, respectively) were analyzed in: 156 CHC subjects; 45 with cirrhosis; 34 CHC responder (CHC R) and 46 CHC non responder (CHC NR); 22 PNAL patients. TNF-α 308 GG homozygous individuals were classified as “low-TNF producers”; TNF-α 308 A carrier individuals as “high-TNF producers”; IL-10 -1082 A carrier individuals as “low-IL-10 producers”; IL-10 -1082 GG homozygous individuals as “high-IL-10 producers”. A significant decrease of the low-TNF-α /high-IL-10 producer genotype *vs* all the other genotypes was observed in Cirrhotic group as compared with CHC subjects (P=0.011). In CHC NR patients the high-TNF-α/high-IL-10 producer genotype *vs* the other genotypes increased significantly as compared with CHC R patients (P =0.048). Finally, the arranged genotype distribution did not significantly differ between PNAL and CHC groups. Conversely, the frequency of TNF-α A allele (high producer) is significantly increased (P=0.048) in PNAL subjects. Our results suggest that: PNAL patients seem characterized by TNF-α high producer; the TNF-α high producer/IL-10 low producer seems characterizing cirrothic patients; the TNF-α high producer/IL-10 high producer seems influencing the negative response to antiviral treatment.

P0960. Interleukin 12B and interleukin 18 gene polymorphisms in women with recurrent spontaneous abortion

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According to Th1/Th2/Th3 immune responses, Th1 related cytokines have been associated with pregnancies resulting in miscarriage, while the production of Th2/Th3 type cytokines has been associated with successful pregnancies. We conducted a case-controlled study to determine the association between the Th1 cytokine gene polymorphisms, such as interleukin 12B (IL-12B) promoter and interleukin 18 (IL-18) promoter, and the risk of recurrent spontaneous abortion (RSA).

The study group consisted of 100 Caucasian Slovenian women with a history of three or more consecutive spontaneous abortions before the 20th week of gestation, of unexplained aetiology. The karyotypes were normal, at a 500 level band of resolution, in all women and their male partners. The control group comprised 100 age and ethnicity matched women with at least two live births and no history of pregnancy loss. Polymerase chain reactions were performed to analyze polymorphisms in the promoter regions of IL-18 (positions -607 and -137) and IL-12B (4bp insertion).

When following the dominant model, the risk of recurrent spontaneous abortion was higher in carriers of IL-12B allele 2 as compared to IL-12B 4bp insertion allele 1 (p=0.018). No significant differences in frequencies of tested IL-18 promoter polymorphism genotypes and the occurrence of RSA were observed.

Our findings suggest that IL-12B allele 2 promoter polymorphisms might be a risk factor for RSA. Further studies comprising of larger numbers of women with RSA are needed to confirm our findings.

P0961. Inflammatory Genes and Cardiovascular Morbidity

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Inflammation plays a pivotal role in the pathogenesis of cardio- and cerebrovascular disease. We studied genetic variations in genes of cytokines, which play a pivotal role in the inflammatory cascade and influence the vascular wall: interleukin-6 (IL-6), interleukin-4 (IL-4), transforming growth factor β1 (TGF-β1), and tumour necrosis factor

α (TNF-α).
The study is part of the Rotterdam Study (n=7983). Multiple polymorphisms were genotyped in each subject. Various phenotypes were studied including clinical end-points such as myocardial infarction (MI), coronary heart disease (CHD), stroke, and early pathology such as atherosclerosis (intima-media thickness and aortic calcifications) and arterial stiffness (pulse wave velocity and distensibility coefficient), and in addition inflammatory markers such as IL-6 and C-reactive protein (CRP). The associations between genotypes and phenotypes were investigated using Cox proportional hazards analyses and analyses of variance. All analyses were adjusted for age, sex and common cardiovascular risk factors.
Genotype and allele proportions were in Hardy Weinberg equilibrium. Only IL-6 and TGF-β showed evidence for a relation with vascular phenotypes. TGF-β1 polymorphisms -509 C/T (p=0.01) and codon 10 Leu/Pro (p=0.02) were associated with risk of stroke, and with arterial stiffness (-509 C/T, p=0.04), but not with MI. IL-6 -174 G/C was associated with arterial stiffness (p=0.03) and levels of CRP (p<0.01), however, it was not associated with MI or CHD.
Of the genes studied only TGF-β1 polymorphisms showed a consistent association with stroke and arterial stiffness. Also the IL-6 polymorphism was associated with early pathology (arterial stiffness).

P0962. FVIII Gene Mutation Profile of High Responder Hemophilia A Patients In Turkey

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FactorVIII (FVIII) replacement therapy is ineffective in hemophilia A patients who develop inhibitors against FVIII. The type of FVIII gene mutation, genes in the Major Histocompatibility Complex loci and also other proteins participating in the presentation of antigens are the major predisposing factors for inhibitor formation. A national collaborative effort was undertaken to identify the FVIII gene mutations in high responder inhibitor patients. Twenty-six severely affected patients with known inhibitor levels of >5 Bethesda U/ml were included. Initially the samples were screened for intron 22 inversions by Southern blotting and then for intron 1 inversions by PCR. The exonic regions corresponding to A2, C2, and A3 domains that include functional binding sites of the ligands of the FVIII protein were then sequenced in inversion negative patients. Complete sequencing of the FVIII gene was performed in those patients without a change in the former exonic regions. About 54% of patients had inversion mutations mostly involving intron 22, and 26% had point mutations that involved 3 single nucleotide and 2 dinucleotide deletions, and 3 transitions in exons corresponding to A2, C2 and A3 domains leading to either nonsense or frameshift and termination mutations, all resulting in protein truncations. One patient had a nucleotide change resulting in a splicing error. Five patients had large deletions of variable length. In conclusion, large genomic rearrangements and termination mutations all leading to a defiency of the FVIII protein were the prominent cause of the inhibitor development in this group of high responder hemophilia A patients.

P0963. Design and validation of DNA pools in an ischaemic stroke population

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Background and Aim: Stroke is a complex, multifactorial disease thought to involve multiple genes of small effect. To identify these, large-scale association studies involving the systematic screening of numerous samples at hundreds of markers are required. This is expensive, time-consuming and labour intensive. DNA pools provide a way to quickly and reliably screen case and control samples in a cost-effective manner by significantly reducing the quantity of DNA used, number of reactions and processing time. The aim of this study was to design and validate DNA pools of stroke cases and healthy controls from Scotland, which could be used to screen for association with single nucleotide polymorphisms (SNPs) using DNA chips.
Methods: Using TOAST criteria, 373 stroke cases were sub-divided into large and small vessel disease pools. Equimolar amounts of DNA from each sample were combined to form 3 pools of over 100 samples each. DNA from 566 control patients was grouped in a similar way into 3 pools. Each had approximately equal proportions of males and females. Allele frequencies at 5 SNP loci were determined for each individual and pool using dynamic allele-specific hybridisation (DASH) and Pyrosequencing.
Results: Allele frequency estimates between pool replicates were consistent for each SNP (average r^2=0.950), and correlated well with allele frequencies determined by individual genotyping (r^2=0.950). Further, alleles were detectable at a frequency as low as 2.5%. These results indicate the validity of these pools as an effective means by which to screen our samples for associations between SNP marker and disease status.

P0964. Localization of candidate regions for a novel gene for Kartagener syndrome

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Asymmetric positioning of internal organs is a characteristic of vertebrates. The normal left-right anatomic positioning, situs solitus, sometimes does not occur normaly, leading to laterality defects. Studies in animal models have shown that laterality decisions are mediated by a cascade of genes that lead to the asymmetric expression of Nodal, LEFTA, LEFTB and PITX2 in the lateral plate mesoderm. Search for mutations in genes implicated in left-right patterning in animal models allowed to identify genes associated with heterotaxia defects in humans. However, these genes explain only a small percentage of human situs defects suggesting that other genes must play a role.
In this study, we report a consanguineous family of Turkish origin, composed of two unaffected parents and three children, two of whom presented Kartagener syndrome. On the basis of the family history, we hypothesize autosomal recessive mode of inheritance. Genotype analysis with polymorphic markers did not show linkage with any known genes or loci causing laterality disorders. Array CGH did not detect a duplication or microdeletion greater than 1 Mb as a possible cause either. Genome wide screening using 10K Affymetrix SNP chips was performed, allowing the identification of two regions of autozygosity, one in chromosome 1 and the other on chromosome 7. In the chromosome 1 locus, a strong candidate gene, encoding the kinesin associated protein 3 (KIF3AP), was not mutated based on SSCP/heteroduplex analysis and direct sequencing. These data provide a basis for the identification of a novel gene implicated in Kartagener syndrome.
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P0965. The keratosis linearis with ichthyosis congenita and sclerosing keratoderma (KLICK) disease gene maps to chromosome 13q

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The erythrokeratodermas is a heterogeneous group of inherited disorders characterized by well-demarcated erythematous lesions and hyperkeratotic plaques. Keratosis linearis with ichthyosis congenita and sclerosing keratoderma (KLICK [MIM 601952]) syndrome is a rare autosomal recessive keratinizing (keratinisation?) disorder. The patients feature palmoplanar keratoderma and linear hyperkeratosis of the skin without evidence of Koebner phenomenon. Some of the

symptoms - massive keratoderma, pseudoainhum and star fish like keratotic extensions resembles those of Vohwinkel syndrome without hearing loss. A deficiency in the formation of keratohyaline granules has been suggested as the cause of KLICK. We have analysed five families with KLICK syndrome of which three families are from Sweden, one from Spain and one from the Netherlands. A 10k SNP array (Affymetrix) was used for autozygosity mapping of the Spanish and Swedish families to map homozygous regions in affected family members. A large region defined by 62 consecutive homozygous SNPs on chromosome 13q was found in one family. The KLICK candidate region was further delineated when combining results from SNP array analysis and highly polymorphic microsatellite markers in all five families A minimal shared homozygous region of 1.3 Mb was identified as associated with KLICK and a search for candidate genes within this region is in progress.

P0966. The supernumerary X chromsome and phenotypic variation in Klinefelter's syndrome

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Klinefelter's syndrome (KS), one of the most common chromosome aneuploidies, has a well-recognised phenotype including small firm testes, aspermatogenesis, high gonadotropin levels, low normal testosterone levels and possibly behavioural and neurological problems. The phenotype is, however, highly variable, and many cases are thought to be undiagnosed. Several features of the X-chromosome may contribute to this variation, including parental origin of the supernumerary X, skewed X-inactivation, and the length of a microsatellite repeat in the androgen receptor (AR) gene that negatively correlates with AR function. We have investigated the effects of these features in 14 47XXY boys for whom detailed phenotypic information is available from their pre-pubertal period onwards. The presence of a paternal X chromosome (n = 3) delayed the onset of puberty, as measured by clinical markers such as increase in serum luteinising hormone concentrations and pubertal acceleration of height growth, by 1.3-1.9 years. In the $47X^{M}X^{M}Y$ boys, longer AR alleles were associated with delayed reproductive hormone level increases, but not with other measures of puberty onset. No phenotypic differences were detected between boys with (n=2) and without (n=4) skewed (>80%) X-inactivation. To our knowledge, we have conducted the first longitudinal study of X chromosome-associated effects on the KS phenotype. Although our sample size is small, our results clearly show that features of the X chromosome contribute to the delay of the onset and progression of puberty in KS boys, which has important implications for the treatment of KS as well as our understanding of pubertal changes in general.

P0967. Locus and gene analysis in a novel autosomal recessive leukodystrophy

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We describe a novel autosomal recessive leukodystrophy in a large Anatolian consanguineous family, displaying neuropathy both in the central and peripheral nervous system. Characteristic clinical features include bilateral cataract at birth, early-onset slow progressive loss of motor abilities and generalized hypotonia more pronounced in lower extremities and distal muscles. Electron microscopy pointed out to a sensory-motor demyelinating peripheral neuropathy. Mental examination revealed normal intelligence. A genome-wide scan followed by further genotyping in the candidate locus identified linkage to 7p15.3-14.3. The maximum two-point lod score obtained was 3.70 at locus D7S2496 (MLINK) and the peak for multi-point lod score was 4.41 between the loci D7S2510 and D7S492 (SIMWALK), encompassing a 9 Mb gene region maximally. Ninety genes map to this region including 3-hydroxyisobutyrate dehydrogenase (HIBADH, GeneID: 11112). All 8 coding exons and exon-intron junctions of HIBADH have been screened by SSCP and DNA sequencing, since 3-hydroxybutyric acid level was found to be elevated in the urine of the proband.

P0968. Linkage analysis on a highly inbred multi-generational pedigree segregating neurological and psychiatric disorders

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We have collected DNA and genealogy information from 96 individuals from a multi-generational, highly inbred pedigree of Gypsy settlers in a rural region of Bulgaria, totaling more than 300 individuals. They come originally from Turkey and their main occupation has been livestock dealers. The genealogy could be traced back up to six generations and the pedigree has been developing with a high degree of interbreeding. A rare Mendelian disorder (spastic paraplegia), as well as major psychiatric disorders (Schizophrenia and Affective Disorders) segregate in this family. We designed a linkage study using Illumina Linkage IV SNP genotyping platform, which interrogates over 5,000 SNPs across all chromosomes. Merlin and Simwalk2 software were used for linkage analysis. A high number of inbreeding loops are found on every genealogical level which poses difficulties when designing the linkage analysis. So far, analyses have been done on a nuclear family segregating autosomal recessive spastic paraplegia. Surprisingly, no conclusive linkage was identified, but suggestive linkage to Chr 2, 12 and 15, it being followed-up with densely spaced microsatellite markers.

P0969. Sign-dependent sampling variance of the linkage disequilibrium measure D'

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The measurement of the extent of linkage disequilibrium (LD) plays a central role in the characterization of multilocus genetic systems at the descriptive and interpretative levels a well as to refine the location of disease genes. D' is one of the most commonly used measures of the extent of LD between pairs of diallelic loci. Previous investigations indicate that the asymptotic sampling variance of D´depends on the sign of deviation from random association. However, sign-dependent sampling variation of D' under experimental conditions is yet little known. Sign-dependent sampling variance of D' was investigated considering a wide range of allele frequencies at the loci, different levels of positive and negative LD intensities, and sample sizes commonly used in experimental studies. Coupling haplotypes were designated are those carrying the most and the least frequent allele variants. Simulations show that empirical sampling variances of D' are considerably larger for negative than for positive LD when allele frequencies at one or both loci are different from 0.5. The present investigations suggest that the precision in the estimation of the extent of LD by D' is substantially improved, under experimental conditions, whether only positive deviations from random association are taken into account.

P0970. Characteristics of singleton SNPs in the human genome and implications for genome-wide association studies

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The human genome is estimated to contain one single nucleotide polymorphisms (SNPs) every 300 base pairs. The presence of LD between SNP markers can be used to save genotyping cost via appropriate SNP tagging strategies, whereas absence or low level of LD between markers generally increase genotyping cost. It is not uncommon that a large proportion of tagging SNPs in a tagging scheme often turn out to be singleton SNPs, i.e. SNPs that only tag themselves rather than contribute power to the rest of a region. If genotyping cost is the primary concern, which may often be the case in the present time for genome-wide association studies, these singleton tagging SNPs would be the primary targets to be removed from genotyping. It is important, however, to understand the characteristics of such SNPs and estimate the impact of removing them in a study. Using the HapMap genotype data, we assessed the distribution and functional implications of singleton SNPs in the human genome. Our results demonstrated that singleton SNPs are not necessarily rare and they potentially can be functionally important (e.g. as nonsynonymous SNPs and SNPs in highly conserved regions). We further assessed

whether these singleton SNPs can be tagged using haplotypes of other non-singleton SNPs in the same regions, and discussed the general implications on genetic association studies.

P0971. Haplotypes in the apolipoprotein L gene cluster are associated with plasma lipids

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The recently characterized apolipoprotein L (apoL) gene cluster on chromosome twenty-two spans approximately 619 kb and contains six genes (apoL-I to apoL-VI). To date, despite the potential to elucidate mechanisms affecting plasma lipid levels, this cluster has not been explored on a genetic level in humans. In this study, 200 unrelated spouses from the Erasmus Rucphen Family (ERF) study were genotyped for 31 single nucleotide polymorphisms (SNPs) spanning the ApoL cluster (approximate average inter-marker spacing of 20kb). Fasting plasma lipids were determined by spectrophotometric chemical analysis. Individual SNPs were analysed with ANOVA. Linkage disequilibrium blocks (n = 6) were estimated with HaploView; haplotypes were inferred and analysed utilizing haplo.stats. Marginal associations were observed ($0.05 < p < 0.10$) for several individual SNPs. Haplotypes in block one and two were associated with total cholesterol (TC)/high-density lipoprotein cholesterol (HDL) ratio ($p = 0.03$ for both). Block four haplotypes were associated with low-density lipoprotein cholesterol ($p = 0.02$). Variation in block five was significantly associated with HDL levels ($p = 0.01$). Haplotypic variation in block six was associated with hypercholesterolemia ($p = 0.03$). These results suggest that the genes in the ApoL cluster play an important role in determining circulating lipid levels, particularly HDL and those measures dependent on it (TC/HDL ratio and affection). Further study of this comparatively unknown genomic region is warranted, particularly with larger sample sizes and denser genotyping.

P0972. An audit of a diagnostic service for LongQT Syndrome.

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Since June 2003 the Oxford Genetics Knowledge Park, UK, has funded a molecular genetic service for LongQT Syndrome. Mutation screening by dHLPC and sequencing is available for the *KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2* and *SCN5A* genes. Since 2003 we have identified pathogenic variants in 57/92 patients referred with a suspected diagnosis of LongQT Syndrome. In 6 probands, more than one potential disease-causing variant was identified; family studies were initiated to investigate pathogenicity. *SCN5A* gene screening has been performed in 6 patients referred with a suspected diagnosis of Brugada Syndrome; no pathogenic mutations were identified.
Mutation testing has been undertaken in 196 relatives, of whom 54% were found to have the familial mutation.
A audit of the analysis performed and the mutations and polymorphisms identified will be presented.

P0973. LRRK2 Gly2019Ser mutation and Parkinson's disease in Italian population

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The Gly2019Ser mutation of the leucine-rich kinase2 (LRRK2) represents the commonest known cause of genetic parkinsonism. It was founded both in families with PARK8-linked autosomal dominant parkinsonism (5-6%) and in sporadic patients (2%) suffering from Parkinson's disease (PD), in different populations.
We screened for Gly2019Ser 38 unrelated Italian familial PD patients with autosomal dominant transmission and 450 sporadic PD patients. Age at onset was 30-78 years. Moreover, we genotyped 180 unrelated healty subjects.
Two familial Pd patients (5.2%) carried Gly2019Ser substitution. One of them was heterozygous, the second one was homozygous and had an affected sibling showing the same rare genotype.
Moreover, 12 (2.7%) sporadic PD patients were heterozygous. The mutation was absent from the control population. Clinical features of both mutation carriers and non-carriers were similar.
The LRRK2 gene product is a member of ROCO protein family, with multiple functional domains, colloquially named dardarin. The Gly2019Ser substitution changes a highly conserved residue of the DYG motif, at the start of kinase activation segment, probably causing an impairment of catalytic activity. To understand this pathological mechanism could help for individuation of new therapeutic strategies.
We confirm that LRRK2 Gly2019Ser is, at the date, the most frequent causative mutation in Italian PD patients, likewise in other populations. A reduced penetrance might account for the presence of the mutation in apparently sporadic PD patients. Moreover, the age-at-onset heterogeneity of mutation carriers suggests that different factors contribute to disease evolution.

P0974. Atypical presentations of hereditary lymphedema type I associated with mutations in VEGFR3

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Genetic studies have identified mutations in the vascular endothelial growth factor receptor 3 gene, VEGFR3/FLT4, in some families with hereditary lymphedema type I (Milroy disease; MIM 153100). Individuals carrying a VEGFR3 mutation exhibit congenital edema in lower limbs, usually bilaterally and below the knees, sometimes associated with cellulitis, prominent veins, papillomatosis, upturned toenails, and hydrocele (in men). In this study, we report atypical presentations of hereditary lymphedema type I in four families in which we identified a VEGFR3 mutation. The clinical findings in two families included spontaneous resorption of lymphedema and elephantiasis. In the third family, the index case presented prenatally with bilateral leg edema, bilateral hydrothorax and lung hypoplasia at 22 weeks' gestation. In the fourth family, the proband was a sporadic case of congenital lymphedema carrying a de novo VEGFR3 mutation. This is the first report of a de novo mutation in VEGFR3.
We conclude that mutations in VEGFR3 can cause more generalized lymphatic dysfunction, or when limited, can undergo spontaneous resorption. Genetic counseling, as well as serial antenatal follow-up, is necessary in families with hereditary lymphedema type I. Moreover, de novo mutations may be present in patients without family history of congenital lymphedema. (http://www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be)

P0975. Haplotype-segregation analysis using three polymorphic markers in *FBN1* gene in patients with Marfan syndrome

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Marfan syndrome (MFS) is an autosomal dominant disorder of connective tissue characterized by caused by mutations in fibrillin-1 gene (*FBN1*) in more than 90% of cases. Widespread use of direct mutation analysis for presymptomatic diagnosis of MFS is challenged because of large gene size and low rate of mutation detection. The alternative is to use haplotype-segregation analysis in family screening which allows determining haplotypes co-segregating with disease in each relative. In our work we used three previously described intragenic microsatellite polymorphic markers MTS-1, MTS-2 and MTS-4. We analyzed 29 families with MFS and 50 unrelated unaffected control subjects from different ethnic groups. 24% of families has been defined as sporadic cases since did not have an autosomal dominant inheritance. We haven't found any significant differences in haplotype frequency distribution between different ethnic groups as in patients so in unaffected individuals. 51 distinct haplotypes were observed on chromosomes of healthy donors. The most common haplotype was 138-145-116 (numbers are given according to allele sizes) which was predominant on normal chromosomes of affected individuals.

19 haplotypes have been determined on mutant chromosomes, 15 of them haven't been found on normal chromosomes. Haplotype frequency distribution on normal and mutant chromosomes were significantly different (χ^2=32.53, df=21, p=0.027). Haplotype 138-163-116 was observed in 18% of cases on mutant chromosomes and in 4% of cases on normal chromosomes (χ^2=6.34; df=1, p=0.012). These data demonstrate possibility and application of haplotype-segregation analysis with use of these intragenic markers for diagnostic purposes in affected families by Marfan's syndrome.

P0976. The association analysis of *SLS6A4*, *HTR2A* and *HTR1B* genes in major depressive disorder

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Major depressive disorder (MDD) is a severe psychiatric disorder with a lifetime prevalence of about 15%. The importance of the genetic component is well accepted, but the mode of inheritance is complex and non-Mendelian. A line of evidence suggests the involvement of serotonin and dopamine neurotransmitters in the pathophysiology of depression. In the present study 94 MDD patients and 227 healthy controls from Bashkortostan (Russia) were genotyped for some polymorphisms in three serotonergic candidate genes: the serotonin transporter, *SLC6A4* (*Stin2 VNTR* and *5HTTLPR* polymorphisms), serotonin 2A receptor, *HTR2A* (*A1438G* polymorphism) and serotonin 1B receptor, *HTR1B* (*G861C* polymorphism, using PCR technique. We found significant differences in *SLC6A4* genotype frequencies (chi2=7.42, P=0.01) between patients and controls. There were increase of the *Stin2*10/*12* genotype (chi2=14.47, P=0.001, OR=2.65) and decrease of the *Stin2*10/*10* genotype (chi2=5.69, P=0.02, OR=0.34) frequencies in depressive group compared to those in control group. *HTR2A*G/*G* genotype (chi2=6.52, P=0.01, OR=1.88) and *HTR2A*G* allele (chi2=5.82, P=0.02, OR= 1.55) are associated with increase risk of MDD. There were no statistical differences between MDD patients and healthy controls in the genotypic and allelic distribution of the *HTR1B* polymorphism investigated.

P0977. Analysis of AZF microdeletions in infertility males.

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Males' infertility in 10-15% of cases is determined genetically and caused by spermatogenesis disturbances. The frequency of AZF loci microdeletions of Y- chromosome is approximately 1:1000-1500 males. We examined 76 patients with infertility, registered in Reproduction and Genetic Consulting Center of Bashkortostan Republic. All patients were divided into 3 groups, based on the semen parameters. The first group included 37 patients with azoospermia, the second - 35 males with severe oligozoospermia (with a sperm count < 5x10(6) million/ml), the third - 4 patients with asthenoteratozoospermia. The control group consisted of 150 fertile males with normal semen parameters. Multiplex PCR-analysis, offered by Simoni et al., 1999, was used for detection of deletions. Deletions of AZF regions were revealed in 8 patients (10.5%) with males' infertility. The frequency of microdeletions was 10.8% in patients with azoospermia, 11.4% - in patients with severe oligozoospermia. 6.58% of all patients had AZFc region deletions, 1.32% - AZFb and AZFc regions deletions, 2.63% - microdeletions of all the three regions. Microdeletions of AZFa+b+c loci were detected in patients with oligozoospermia, that conflict with the results of other scientists that can be explained by either semen analysis inaccuracy or gonadal mosaicism existence. Y chromosome microdeletions in control group of fertile males were not found. The results showed that deletions frequency was higher than average frequency in other world populations, where it is approximately 7.5%.

P0978. Partial AZFc deletions with loss of CDY1b represent a risk factor for male infertility among Macedonians

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Complete deletions of AZFc region in the distal Yq are the most frequent genetic cause of male infertility. Partial deletions within the AZFc region have also been described, but their contribution to male infertility is still unclear. Our objective was to study the occurrence of partial AZFc deletions among men from the Republic of Macedonia. We studied 200 infertile/subfertile males with different spermatogenic phenotype and 241 fertile controls (proven fathers) using AZFc specific sequence tagged site (STS) markers and DAZ and CDY1 specific single nucleotide variants (SNV). We identified 18 men with partial AZFc deletion; 11 in the group of infertile/subfertile patients (11/200; 5.5%) and 7 among fertile controls (7/241; 2.9%). Based on the STS analysis, we found two types of partial AZFc deletions; 14 gr/gr and 4 b2/b3 deletions. Both types were found in men with different spermatogenic failure and in fertile controls. The frequencies of deletions that lack DAZ1/2 and DAZ3/4 genes were similar in infertile/subfertile men and fertile controls. Loss of CDY1b SNV was found only in men with partial AZFc deletion. The frequency of deletions with loss of CDY1b was significantly higher in the infertile/subfertile group (9/200; 4.5%) in comparison to the fertile controls (2/241; 0.8%) (p=0.014; OR=5.63; 95% CI: 1.20 to 26.37). Our data suggest that partial AZFc deletions involving loss of CDY1b represent a risk factor for male infertility among Macedonians.

P0979. The Mannose-Binding Lectin Gene Polymorphisms In Patients With Chronic Hepatitis B And Spontaneously Recovered :A Case-Control study

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Background: Mannose-binding lectin (MBL) is a constituent of the human innate immune system which may play an important role in combating a variety of infectious diseases and thus may be important for determining hepatitis B virus (HBV) persistence. In this study, we determined MBL genotypes in chronic hepatitis B subjects, spontaneously recovered subjects and healthy controls.

Methods: In a case-control study, we examined 100 unrelated patients with Chronic hepatitis B and 100 spontaneously recovered patients were referred to Taleghani Hospital and 100 healthy controls which all had been matched by sex and age. MBL gene polymorphisms were determined by PCR-RFLP methods.

Results: The MBL genotype distributions in the three groups are shown in Table. Our finding have shown that the frequency of -550C, -221G, +4T, +52T, +54A and +57A alleles were 0.57, 0.76, 0.135, 0.22, 0.22 and 0.05 in chronic HBV patients; 0.57, 0.80, 0.175, 0.37, 0.3 and 0.05 in spontaneous recovered subjects, respectively. Our results show that there is no association between each polymorphisms of the MBL gene with persistent HBV infection in Iranian population excepting codon +52 (p=0.0).

Conclusion: Heterozygous for codon 52 mutant allele (C/T) may be associated with recovery from acute hepatitis B.

Table .Distribution of MBL genotypes in the studied population

Polymorphisms	Chronic HBV patients N (%)	spontaneous recovered HBV N (%)	Healthy controls N (%)
-550 G/G	18	15	23
-550G/C	50	54	37
-550C/C	32	31	40
-221C/C	4	5	12
-221C/G	40	29	27
-221G/G	56	66	61
+4 C/C	74	68	75
+4 C/T	25	29	24
+4 T/T	1	3	1
+52C/C	67	27	39
+52C/T	22	71	57
+52T/T	11	2	4
+54G/G	63	52	48
+54G/A	29	36	40
+54A/A	8	12	12
+57G/G	99	99	90
+57G/A	1	1	9
+57A/A	0	0	1

P0980. A protocol for semiautomatic detection of mutations associated with thoracic aortic aneurysms/dissections and Marfan Syndrome

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OBJECTIVE Mutations in the Fibrillin-1 gene (FBN1) are the major cause of Marfan Syndrome (MfS) and are also associated with isolated aneurysms/dissections. The objective of this study is to establish a semiautomatic protocol for rapid sequencing of both Marfan genes, FBN1 and TGFBR2.

METHODS Main features of the protocol are: i) successive analysis of individual patient samples ii) optimized design of primer pairs allowing uniform conditions for both PCR amplification and sequencing iii) implementation of a liquid handling robot to achieve semiautomatic performance of all pre- and post-PCR steps.

RESULTS The protocol was validated by testing 25 consecutive patients refered to hospital for ectomy of an aneurysm/dissection of the ascending aorta. 15 patients (60%) also showed other signs of MfS. A mutation in FBN1 could be identified in 12/25 patients (48%). 9/12 mutations were novel (75%). 67% of the patients with suspected MfS showed a FBN1 mutation, while only 20% of the patients with no further signs of MfS carried a FBN1-mutation. No alteration in the FBN1 coding sequence could be found in 13 out of 25 patients (52%), two of which were later found to harbor a mutation in TGFBR2 (8%).

CONCLUSION The novel protocol enables rapid detection of the disease causing mutation in the index patient. This may in turn pave the way for timely identification of pre-symptomatic relatives who should then be monitored closely.

P0981. Identifying the gene responsible for medullary cystic kidney disease type 1

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Medullary cystic kidney disease (MCKD) is an autosomal dominant chronic tubulointerstitial nephropathy. It is similar to nephronophthisis, the main difference being the mode of inheritance and the later age of onset of MCKD. The disease is characterized by thickening of the renal tubular basement membrane, renal fibrosis and hypertension however there is great clinical heterogeneity. At least three loci have been linked to MCKD: 1q21 with MCKD1, 16p12 with MCKD2 and a third unknown locus with MCKD3. The gene responsible for MCKD2 has been found to be *Uromodulin* whereas the gene responsible for MCKD1 is still unknown. The purpose of this study is to identify and characterize the gene involved in MCKD1. The initial critical interval containing the responsible gene, spanned a gene-rich 8cM region. Further linkage analysis using more kindreds have reduced the critical region. Recently a comparison of haplotype blocks of affected individuals from several kindreds of different geographic origin (Wolf et al, Kidney Inter. 2003, 64(3):788-92) reduced the area to 650kbp. We used a bionformatics approach to select some good candidate genes to analyse. Direct exon sequencing of protein inhibitor of activated STAT protein 3 (PIAS3), interleukin receptor 6 (IL6R), apolipoprotein 1 (APOAIBP), carbonic anhydrase XIV (CA XIV) and kidney predominant protein (NCU-G1) have revealed no causative mutations. Finally, after the identification of several recombinants we are using microsatellite marker analysis to further reduce the critical region.

P0982. *RET* variants and haplotypes in the context of Multiple Endocrine Neoplasia type 2.

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Multiple endocrine neoplasia type 2 (MEN 2) is an autosomal dominant cancer syndrome, which is divided into three subtypes: MEN 2A, MEN 2B and familial medullary thyroid cancer (FMTC). Approximately 92% of MEN 2 cases are caused by mutations in exons 10,11,13-16 of the *RET* proto-oncogene. There exists inter- and intra-familial phenotypic variability among the MEN 2 families, even though when the disease is caused by the same *RET* mutation, suggesting a role for genetic modifiers, such as polymorphisms/haplotypes.

We have sought to determine the frequency and position of *RET* germline mutations in a cohort of 114 Spanish probands with any sign of MEN 2, and to search for putative modifier loci. With this aim, the distribution of 8 *RET* polymorphisms and the haplotypes comprising them, were subsequently studied in the context of the families positive for *RET* mutational screening. The relationship between *RET* mutation type and presence of a polymorphism/haplotype was analyzed. It was also studied the relationship between the presence of pheochromocytoma (PC) and/or hiperparathyroidism (HPT) in carriers of the same *RET* mutation, and the genotype for the specific variants. The results derived from those analyses revealed no associations of any variant/haplotype to a specific mutation or to the clinical presentation. Nevertheless, these observations do not permit us to exclude the possible role of other variants in *RET* or another related genes, in the final presentation of the disease. Supported by Fondo de Investigación Sanitaria, Spain (PI040266 and Red de Centros INERGEN C03/05).

P0983. A Meniere's disease gene linked to chromosome 12p12.3

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Meniere's disease (MD) is characterised by spontaneous attacks of vertigo, fluctuating sensorineural hearing loss, tinnitus and aural fullness. The majority of patients with MD appear sporadic but 5-13% of the cases have a family history for the disease. The cause of both the sporadic and inherited forms of MD remains unclear despite a number of candidate genes defined from their association with hearing loss.

We have performed a genome wide linkage scan on a large Swedish family segregating MD in five generations. Two additional families with autosomal dominant MD were analysed for linkage and a cumulative Zmax of 3.46 was obtained for a single region on chromosome 12p. In two of the three families, a shared haplotype was found to extend over 1.7 Mb suggesting a common ancestral origin.

P0984. Analysis of Alu insertion polymorphisms in the DSCAM, LAMA2 genes in children with mental retardation in Volgo-Ural region of Russia

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Mental retardation (MR) is a developmental disability, characterized by a global deficiency in cognitive abilities, an inability to cope with every day life and an onset during childhood. Down syndrome cell adhesion molecule, encoded by the *DSCAM* gene located on chromosome 21q22.2, is a novel member of Ig superfamily of cell adhesion molecules and play central role in the development and plasticity of the human central and peripheral nervous systems. The *LAMA2* gene located on chromosome 6q22-q23, coding for the Laminin, a heterotrimeric extracellular matrix protein, is involved in the developing brain.

This study reports the results of Alu insertions analysis of the *DSCAM*, *LAMA2* genes in male patients of 3 to 18 years with non-specific MR (n=214) and healthy donors (n=217) from different ethnic groups of Volgo-Ural region of Russia. We analysed the distribution of I/D alleles of these DNA-loci and found statistically significant differences between MR patients and healthy donors in the *DSCAM* and *LAMA2* genes. The Alu-insertion *Yb8106* in the *DSCAM* gene was associated with an increased risk of MR in Russans (OR=1.79, CI% 1.05-3.03). There were essential distinctions in the distribution of allele and genotype frequencies between MR patients with different level of MR. There was evidence of association between the Alu-insertion *Ya5491* in the *LAMA2* gene and MR in Bashkirs (OR=1.34, CI% 1.03-1.89).

This study suggested that the Alu insertion polymorphisms in the *DSCAM*, *LAMA2* genes could be valuable for assessment of MR risk.

P0985. BDNF Val66Met and Psychiatric Disorders: Meta-Analysis of Case-Control Studies confirm association to substance-related disorders and eating disorders

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There is an increasing recognition that the pathophysiology of mental disorders could be the result of a local deregulation of synaptic plasticity, and a possible explanation would be an altered synthesis and/or release of neurotrophins. The Val66Met SNP, located in the pro-BDNF sequence has been extensively studied through linkage and association approaches in several psychiatric disorders. The purpose of our study was to test if genetically driven variation of BDNF may significantly be involved in a non illness-specific susceptibility to mental disorders or, alternatively, a certain group of disorders may be strongly related to this gene but others can result from spurious association studies. To test this hypothesis, we performed a meta-analysis restricted to individual case-control studies in different categories of mental disorders. This meta-analysis includes data from 30 case-control studies and five psychiatric phenotypes: eating disorders, substance-related disorders, mood disorders and schizophrenia. We have found an overall gene effect on psychiatric disorders as a single group. From the stratified analysis according to diagnostic categories, we have found that in the case of mood disorders (both in a global analysis and in another one only considering bipolar patients) and schizophrenia, the Val66Met polymorphism in BDNF is not a risk factor. Subtype analyses also indicate that the association of the Val66Met polymorphism in BDNF is confined to two particular diagnostics: substance-related disorders and eating disorders. Our results are suggestive of a protective effect of Met allele of BDNF for substance-related disorders and a risk factor for eating disorders.

P0986. Beta3-adrenoreceptor gene W64R polymorphism in metabolic syndrome patients with type II diabetes.

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Beta3-adrenergic receptor gene W64R heterozygosity has been reported to associate with obesity and other features of the metabolic syndrome (MS) in several ethnic populations. In our study we determined the frequency of WR genotype in MS patients with type II diabetes and in healthy children and adolescents, randomly sampled from Saint-Petersburg population. MS patients with type II diabetes (208 women, 75 men, mean age 59±9), 33 of them (12%) with normal weight (BMI<25 kg/m^2), 96 (34%) overweight (BMI≥25 kg/m^2) and 154 (54%) obese subjects (BMI≥30 kg/m^2), and healthy children (232 girls, 223 boys, mean age 12±3) were genotyped with PCR-RFLP method. Results were analyzed using Chi-square test. We did not find any sex-dependent differences in genotype and allele frequencies distribution between the patients and the controls (p=0.16). At the same time there was a statistically significant difference in genotype and allele frequencies distribution between the patients with BMI≥30 and the patients with BMI≥25 (p=0.01); between the patients with BMI≥30 and the controls (p=0.02). Thus, we found the frequency of heterozygotes was lower in the group of MS patients with type II diabetes and BMI≥30 than in the controls.

	patients			controls		
	men (%)	women (%)	Σ (%)	boys (%)	girls (%)	Σ (%)
WW	65 (86,7)	180 (86,5)	245 (86,6)	182 (81,6)	193 (83,2)	375 (82,4)
WR	10 (13,3)	28 (13,5)	38 (13,4)	41 (18,4)	39 (16,8)	80 (17,6)
W	0,93	0,93	0,93	0,91	0,92	0,91
R	0,07	0,07	0,07	0,09	0,08	0,07

	patients			controls
	BMI<25kg/m2 (%)	BMI≥25kg/m2 (%)	BMI≥30kg/m2 (%)	Σ (%)
WW	30 (90,9)	75 (78,1)	140 (90,9)	375 (82,4)
WR	3 (9,1)	21 (21,9)	14 (9,1)	80 (17,6)
W	0,95	0,89	0,95	0,91
R	0,05	0,11	0,05	0,07

P0987. Genetic polymorphisms in methylenetetrahydrofolate reductase and risk of chromosome 21 non-disjunction

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Aneuploidy is the most commonly identified chromosome abnormality in human. Our knowledge about the mechanisms underlying chromosomal non-disjunction is, however, surprisingly poor. The origin of the extra 21 chromosome is maternal in 95% of cases and due to failure of normal chromosomal segregation during meiosis I. Advanced maternal age is the only fully accepted risk factor for trisomy 21. Other risk factors are not well known. The relationship between chromosomal non-disjunction leading to aneuploidy and folate metabolism has drawn attention in the recent years. In this study, we examined the two polymorphisms in genes encoding the folate metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR) among 39 mothers of children with Down syndrome and 90 age-matched control mothers from West Siberia (Russia). The frequencies of *MTHFR* C677T genotypes (CC, CT, and TT) were 51%, 36% and 13%, respectively, in the case group and 49%, 41% and 10%, respectively, in the control group. No significant differences were found in the genotypes distribution for *MTHFR* C677T variant between the two groups (P = 0.81). The frequencies of *MTHFR* A1298C genotypes (AA, AC, and CC) among case mothers were 31%, 56% and 13%, respectively. The corresponding frequencies among controls were 48%, 43% and 9%, respectively. No significant differences between the two groups were found also (P = 0.28). Our results do not support the link between the C677T and A1298C polymorphisms of *MTHFR* and risk of chromosome 21 non-disjunction in maternal meiosis I. This research was supported by Grant of President of Russian Federation (MK-1969.2005.4).

P0988. Association of the serotonin transporter gene (*SLC6A4*) with migraine with aura in Spanish patients

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Migraine is a complex neurological disorder affecting 8% of men and about 18% of women. Several studies suggest that serotonin-related genes participate in its pathogenesis. Involvement of the serotonin transporter gene (*SLC6A4*) in migraine has been studied in different populations with discordant results. To evaluate the association of *SLC6A4* polymorphisms with migraine in the Spanish population we performed a case-control study considering 91 migraineurs (34 migraine without aura (MO) and 57 migraine with aura (MA) -19 of which were hemiplegic -) and 109 healthy controls. We selected three polymorphisms distributed along the *SLC6A4* gene: 5HTTLPR (a deletion/insertion in the transcriptional regulatory region), 5HTTVNTR in intron 3 and the rs2066713 tag SNP in intron 2. No significant differences were observed for the 5HTTVNTR and rs2066713 polymorphisms. However, the genotype distribution of the 5HTTLPR polymorphism was significantly different between the MA and control groups (p=0.007). Individuals carrying the L allele presented 4-times increased risk of suffering MA than non-carriers (p=0.003). This association remained significant in patients with non-hemiplegic aura (p=0.008 OR=4.7). Interestingly, although these results support the implication of *SLC6A4* in MA, we observed that our group of patients showed an overrepresentation of the 5HTTLPR-L allele instead of the 5HTTLPR-S variant that has been associated with migraine in most of the previously studied populations. This observation, together with

the fact that linkage disequilibrium patterns differ among populations, suggests that the 5HTTLPR polymorphism may not confer susceptibility to migraine, but is in linkage disequilibrium with another variant directly involved in this complex phenotype.

P0989. Contribution of syntaxin 1A to the genetic susceptibility to migraine: a case-control association study in the Spanish population

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Migraine is a common neurological disorder with a complex inheritance pattern. However, mutations in the *CACNA1A* gene, encoding the α1A subunit of the Cav2.1 neuronal calcium channel, have been identified in rare autosomal dominant forms of the disease. This finding suggests that ion channels or related proteins might be also involved in the common forms. Thus, we focused on the genes for syntaxin 1A (STX1A) and SNAP-25 (components of the SNARE complex, which regulates the Cav2.1 channel activity) and performed a population-based association study. We selected two tag SNPs of *STX1A* -rs941298 and rs4363087- and three tag SNPs of *SNAP-25* -rs362990, rs362987 and rs4813925-, and genotyped 91 migraineurs (34 migraine without aura (MO) and 57 with aura (MA)) and 109 healthy controls. We found no significant association between *SNAP-25* and migraine. However, we observed significant differences in both allele frequencies (p=0.003 OR=1.9) and genotype distributions (p=0.01) between migraineurs and controls when the rs941298 SNP of the *STX1A* gene was considered. Once we subdivided patients into MO and MA, evidence of association for markers rs941298 and rs4363087 was detected in the MO subgroup, with an increased number of patients carrying the rs941898T and rs4363087G alleles (p=0.010 OR=2.9 and p=0.016 OR=2.9). We also observed a strong association between the T-G haplotype (rs941298-rs4363087) of *STX1A* and migraine (p=0.007 OR=1.9), still significant when patients were subdivided into MO and MA. Neither epistatic phenomena nor sex influence were detected. Our data suggest a contribution of the *STX1A* gene to the genetic susceptibility to migraine.

P0990. Study of an SNP in *POLG1* gene in patients with hypertension

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Energy balance play important role in the process of cardiovascular system function. Proteins that participate in energy production are encoded by either mitochondrial genome or nucleus. One of chromosomal genes which ensures mitochondrial DNA replication is *POLG1* encoding for mitochondrial DNA polymerase gamma. To investigate association of an SNP in this gene (rs2238296) with endophenotypes of cardiovascular system, a group of hypertensive patients has been investigated (92 individuals, 59 males and 33 females). The mean age in the sample was 47.1 years. All the patients were subjected to echocardiographic examination and daily blood pressure monitoring. Allele C frequency in the sample was 42% and did not differ from population data. By comparing characteristics of blood pressure and ultrasound examination in different genotypes carriers (TT, TC, CC), we found the following associations. In females, the SNP was associated with end diastolic size of the left ventricle and with systolic blood pressure (p<0.05). In males, there was association of homozygous CC genotype with higher levels of maximal blood pressure during veloergometry (p=0.022). Thus, a polymorphism in *POLG1* might be associated with quantitative characteristics of cardiovascular system in hypertensive patients. This intronic polymorphism can be in linkage disequilibrium with functional variants.
The study was supported by RFBR grant 04-04-48532.

P0991. Genotype distribution and allele frequencies of the Methylentetrahydrofolate reductase gene polymorphism (C677T, A1298C) in women with coronary heart disease (CAD) in St. Petersburg, Russia.

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The methylentetrahydrofolate reductase (MTHFR) plays a key role in metabolism of plasma homocysteine. The C677T MTHFR gene polymorphism is accompanied by reduction of enzyme activity and increased homocysteine level.The A1298C polymorphism in exon 7 of the MTHFR gene ads to aglutamate-to-alanine exchange, this variant also decreases MTHFR enzyme activity. The association of the coronary artery disease (CAD) risk in young men group with MTHFR gene polymorphisms was found in many studies.

In 79 angiographicaly diagnosed CAD women and in 145 unrelated controls genotypes of MTHFR C677T and A1298C polymorphism were determined by polymerase chain reaction-restriction fragment length polymorphism analysis. The genotype distribution was in Hardy-Weinberg equilibrium for all variants. Statistical significance of differences between groups was assessed with $\chi 2$ tests.

Genotype distribution and allele frequencies in the controls and the group of patients ($p<0.05$).

We found no significant differences in genotypes and alleles frequency distribution between patients and population control. Our findings neither support nor exclude possible associations between genetic variations in the MTHFR gene and the presence of cardio-vascular disease states.

Genotype distribution and alleles freqencies in the controls and the group of patients

Genotype	Allele	Controls n (%)	Patients n (%)	P-value
677 C C 677 C T 677 T T		93 (62.84) 47 (37.76) 8 (5.4)	41 (51.9) 32 (40.5) 6 (7.6)	0.276
	T	63 (21.28)	44 (27.85)	
1298 A A 1298 A C 1298 C C		68 (49.3) 47 (34.0) 23 (16.7)	29 (40.84) 26 (36.62) 16 (22.54)	0.434
	C	93 (37.7)	58 (40.85)	

P0992. The c.1298A>C polymorphism in the Methylenetetrahydrofolate Reductase (MTHFR) gene is risk factor for Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic inflammation that causes joint deformity, pain, functional limitations and psychological distress leading to work disability and consequent relevant social and individual costs. RA is a complex disease that results from the interplay between genetic and environmental factors. Identification of the genetic factors involved in the pathogenesis of RA could be crucial for early diagnosis and development of treatment strategies directed at the cause of the disease. However, still little is known susceptibility genes for RA. Although HLA-DRB1 is the main RA gene, it accounts for only part of the genetic risk for RA. Association studies suggested that other genes, including TNFR2, PADI4, SLC22A4, RUNX1, and PTPN22 play a role in the etiology of the disease.

Metotrexate (MTX) is the first line treatment for RA and its efficacy has been reported to be dependent on gene variant in the Methylenetetrahydrofolate Reductase (MTHFR) gene. To assess the possible role of MTHFR polymorphisms in the etiology of RA, we genotyped the c.677C>T and the c.1298A>C variant in MTHFR gene in a group of 217 Italian RA patients and in a matched group of healthy

controls. While no significant difference of c.677C>T genotypes between cases and controls was found, a clear increase of c.1298CC genotype was observed among RA patients (OR=2.59; p=0.0093). Our results suggest that MTHFR c.1298CC genotype may be considered a low-penetrance susceptibility factor for RA and point out a possible role of folate metabolism in the etiology of the disease.

P0993. Association of the apolipoprotein E genetic polymorphism and multiple sclerosis risk in Bashkortostan

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Allelic variants of apolipoprotein E (APOE) gene influence the course of many neurological diseases. We investigated whether a polymorphism in the APOE gene is associated with both susceptibility to and clinical characteristics of Multiple Sclerosis (MS). We evaluated APOE gene polymorphism in unrelated 120 Tatar and 132 Russian patients of MS from Bashkortostan and 238 Tatar and 217 Russian healthy subjects from this area. No significant differences were observed in the allelic frequencies of the APOE gene between patients with MS and healthy control subjects in Russian and Tatar cohort. But in Tatar the frequencies E2/E4 genotype and E4 allele was higher in patient MS with coordination disorder in debut then controls (E2/E4 7.69% vs. 0.84%; P=0.009; OR=9.83, CI=1.49-79.75 and E4 23.07% vs. 12.81%; P=0.012; OR=2.04, CI=0.16-3.57). In this group the frequency of E3 allele in patient was significantly lower than in control: 69.23% vs. 80.88% (P=0.013; OR=0.53, CI=0.32-0.87). These results suggest that APOE gene polymorphism is specifically associated with clinical characteristics of current disease.

P0994. Mutation analysis of the 5,10 methylenetetrahydrofolate reductase (MTHFR) gene in multiple sclerosis

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Multiple sclerosis (MS) is a disease that causes demyelinisation on portions of the axons in the brain. During this process transmission along these axons is impeded. Even though MS is such a prevalent disease, affecting 1 in 1000 individuals, the cause(s) of this complex condition is not yet fully understood. Nitrous oxide has been implicated in the onset of MS because of its action in the inhibition of the folate and homocysteine metabolic cycles. 5,10 Methylenetetrahydrofolate reductase (*MTHFR*) is one of the enzymes that plays a role in the folate cycle. The current investigation tested the possible involvement of the *MTHFR* gene in the aetiology of the disease. This case-control study included MS patients, some of whom developed the disorder following exposure to nitrous oxide, and healthy control individuals. Mutation analysis of the *MTHFR* gene was performed using polymerase chain reaction (PCR), heteroduplex single strand conformational polymorphism (HEX-SSCP) detection and semi-automated DNA sequencing techniques. These procedures were applied to identify any known and/or novel variations in the *MTHFR* gene that may be associated with a predisposition to nitrous oxide-induced MS. Two novel polymorphisms, 1056C/T and 1782G/C, and one previously described mutation, 1286A/C, were identified. A statistically significant difference in genotypic frequency was noted between patients and control cohorts, and when comparing patients exposed to N_2O with patients not exposed to N_2O, for the 1782G/C polymorphism (p = 0.03), implicating a possible role for *MTHFR* in developing MS.

P0995. Plasminogen activator inhibitor-1 (PAI-1) genetic polymorphism and its role in the progression of multiple sclerosis.

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Our aim was to test the hypothesis that impaired proteolytic mechanisms in multiple sclerosis (MS), which were previously published to play a role in the evolution of MS, were in part due to the common functional genetic polymorphism in the plasminogen activator inhibitor-1 (PAI-1) promoter 4G/5G. Previously reported data showed defective fibrinolysis in MS with PAI-1 being one of the major enzymes included. The effects of fibrinolytic system are mediated by plasmin, the protease generated by the action of plasminogen activators (PA) on the inactive precursor plasminogen. The major inhibitor of tissue PA is PAI-1. The genetic polymorphism 4G/5G modulates the expression of PAI-1 gene and individuals with the 4G/4G genotype have increased plasma PAI-1 concentrations. PAI-1 4G/5G polymorphism was evaluated in the group of 313 patients with multiple sclerosis (MS) and 376 healthy controls from Slovenia and Croatia. The significance of the difference of observed alleles and genotypes were determined using the $\chi 2$ test. Relationship of the genotypes and the disease progression and the age of onset of the disease was tested by one-way ANOVA test and logistic regression analysis. Our results showed statistically significant differences in the distribution of PAI 4G/5G genotypes with respect to progression of the disease: ANOVA (P<0.01) and logistic regression (P<0.02) analysis. Namely, genotype 5G5G was associated with lower progression index PI, which was calculated as a ratio EDSS/disease duration in years. Our results suggest that reduced capacity for proteinase inhibition may be involved in the slower progression of MS.

P0996. PRKCA shows association to multiple sclerosis in two populations

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Multiple sclerosis (MS) is a chronic inflammatory disease of the CNS. Both unknown environmental factors and genetic predisposition are required to generate MS.

A set of 63 Finnish MS families, originating from a high-risk region of the country, was used to identify a susceptibility gene within the previously established 3.4 Mb region on 17q24. Initial SNP-based association implicated *PRKCA* gene, and this association was replicated in an independent set of 148 Finnish MS families (p=0.0004). Further, 211 SNPs covering the *PRKCA* gene and the flanking regions was analyzed in 211 Finnish and 554 Canadian MS families.

SNP haplotype and genotype combination analyses revealed two allelic variants of *PRKCA* to be over-represented either in Finnish (Odds Ratio: 1.34, 95th CI 1.07-1.68) or in Canadian (Odds Ratio: 1.64 95th CI 1.39-1.94) MS cases. The expression level of *PRKCA*, in CD4 negative mononuclear cells of five Finnish multiplex families and in lymphoblast cell lines of 11 CEPH individuals of Europen origin, correlates with the copy number of the these *PRKCA* risk alleles. Linkage, association and the preliminary functional study of *PRKCA* gene imply it's involvement in the etiology ot the MS.

P0997. HLA class II polymorphism in Myasthenia Gravis patients from Bashkortostan, Russia

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The genetics of the autoimmune response in Myasthenia Gravis (MG) is not well understood, but evidence suggests that human leukocyte antigens (HLA) genes within the major histocompatibility complex may play a role in the development of the diseases. The association of MG with the distinct HLA haplotypes varies between ethnic populations.

HLA-DRB1 gene polymorphism was analyzed in 40 (Russian and Tatar) patients with the generalized form and early onset of myasthenia without thymoma and 106 healthy ethnically matched controls from Bashkortostan, Russia.

The specificities frequencies of HLA-DRB1 polymorphism differed significantly between patient and controls (χ^2=55,2505, p<0,0001) and found to be 32,50% and 8,49% for DRB1*17 (χ^2=24,3199, p=0,0005) and 10,0% and 1,42% for DRB1*16 (χ^2=9,5592, p=0,0029), correspondingly. Consequently, oligotyping has revealed positive associations of HLA-DRB1*17 (OR=5,19, 95%CI 2,52-10,74), and DRB1*16 (OR=7,74, 95%CI 1,80-37,94,) with the disease based on case-control study. The frequency of DRB1*07, which had been considered protective in Russian population, were 7,5% in our patients and 15,57% in controls but differences were not significant (χ^2=2,6061, p=0,1066).
These findings indicate that the immunogenetic backgrounds in our MG patients are heterogeneous and apparently different from those in Caucasian and Asian patients. Comparisons among disease-susceptible HLA class II alleles and clinical manifestations of MG in different ethnic groups would be helpful in determining the pathogenesis of the disease.

P0998. MYH mutations in Belgian polyposis families.

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Individuals with homozygous or compound heterozygous mutations in the base excision repair gene MYH are predisposed to develop multiple colorectal carcinomas and classic adenomatous polyposis. To evaluate the contribution of the MYH-mutations in Belgian polyposis families, mutation analysis has been performed by DHPLC on 179 unrelated patients in whom no mutations were identified in the APC- and MMR-genes or from whom the tumors were microsatellite stable. Six patients were found with biallelic mutations of the MYH gene: p.Y165C/p.Y165C, p.G382D/c.1249-1263del15bp, p.Y90X/p.P391L and, in three patients, p.Y165C/p.P391L. It is remarkable that four of these six patients were compound heterozygous for the p.P391L mutation what suggests a founder mutation. This missense mutation has previously also been found in 14% of the patients with biallelic mutations in the Netherlands (1). The c.1249-1263del15bp mutation has not been reported before. Further, seven patients were heterozygous carriers of only one pathogenic MYH mutation. This observation fits with the emerging evidence that monoallelic MYH mutation confer a potentially low-penetrant risk of colorectal cancer (2). Finally, the presence of four novel missense mutations (p.A208V, p.A226S, p.R295C, p.A359V) in a heterozygous state in the patient group and their complete absence in 200 healthy controls may also add to this feature.
In conclusion: 10 % of the FAP/AFAP phenotypes (6/58) were characterized as MAP (MYH Associated Polyposis) from which more than half were (compound) heterozygous for p.P391L, a potential Belgian/Dutch founder mutation.
(1) Nielsen M et al., J Med Gen 2005;**42**:e54
(2) Croitoru ME et al., J Natl Cancer Inst 2004;96:1631-4

P0999. Spectrum of mutations of *MYO15A* associated with hearing loss gives insight into the function of myosin XVA

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MYO15A is located on chromosome 17p11.2 and encodes unconventional myosin XVA, which is necessary for transduction of sound by inner ear hair cells. Mutations of *MYO15A* are associated with nonsyndromic, autosomal recessive hearing loss DFNB3 in humans and deafness and circling behaviour in the shaker 2 mouse. There are two classes of alternative splice variants of *MYO15A*, with and without exon 2, which encodes 1220 residues that preceeds the motor domain, and hitherto without a known function in the auditory system. We ascertained over 600 families segregating hereditary hearing loss. Among this cohort, there was evidence of linkage of markers for DFNB3 to hearing loss in thirty-eight of these families who were from Pakistan, India and Turkey (Thirty families from Pakistan, six from India and two from Turkey). We report eighteen novel recessive mutations of *MYO15A* segregating in twenty-two of the thirty-eight families. Two homozygous truncating alleles of *MYO15A* are associated with moderate to severe hearing loss and are located in exon 2 indicating for the first time, the necessity of this sequence for the function of myosin XVA in normal hearing.

P1000. Genotype - phenotype correlation in myotonic dystrophy

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Myotonic dystrophy type I- the most common form of muscular dystrophy in adults , affecting 1/8000 individuals .DM is associated with the expansion and instability of a CTG repeat in the 3' untranslated region of the myotonic dystrophy protein kinase gene located on chromosome 19q13.3 The aim of this study was genotype-phenotype correlation in Iranian population. We analyzed a small group of these patients for determination of clinical and genetic characteristic of DM1.
PCR , Southern blot were used to clarify equivocal clinical diagnoses and confirm clinical findings. Diagnosis was based on clinical and Electromyographic. 82 patients registered with DM were reviewed. In 58 patients(70/73 %), we detected one band and 24 patients have no expansion of the repeat (two bands).We studied 25 DM - families, a total of 36 patients who were single band (23 Male, 13 female, mean age 37.4 ± 13.3), of whom 21 were diagnosed with a CTG expansion and the rest of them are in progress.
The mean of normal CTG expansion was (8.6 ± 3). Twenty patients had CTG repeat expansion between 130 and 800, and one of the patients had 97 CTG repeat expansion. We found relation with Muscular Disability Rating Scales and a Sum of Symptoms Score, age of onset and the number of expansion.
Our results proved correlation of the expansion size and muscular disability except for one case with no signs of myotonia. There is no correlation of cataract and endocrine dysfunction and the expansion size in DM1 patients.

P1001. Mutational analysis in Neurofibromatosis type 1 patients: Identification of 19 novel mutations

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Mutations in the NF1 gene are the cause of neurofibromatosis type1, the most common tumor-predisposing disorder in humans. Mutation analysis in the NF1 gene is complicated for several reasons, among which are the large size of the gene and the diversity of pathogenic mutations found. There is no single assay which can detect all NF1-mutations. The majority of NF1 cases, however, are caused by base changes, small deletions and duplications/insertions. As a first step in our strategy for mutation detection we have therefore used a sensitive SSCP assay. In 40 patients with clinically confirmed NF1 all coding sequences of NF1 including the exon/intron boundaries were screened and variants subject to sequence analysis. 7 known and 19 novel mutations considered to be pathogenic were identified in addition to a number of known polymorphisms. Among the 19 novel mutations the entire spectrum of different types was found: 6 missense mutations (c.480G>T, c.581T>C, c,3646G>T, c.3764A>G, c.5407A>G, c.5486T>A), 3 nonsense mutations (c.503C>A, c.706C>T, c.1603C>T), 7 small deletions (c.499_502delTGTT, c.1745G>C;c.1746_1783del, c.2974_2976delATG, c.3186delA, c.3482_3483delTC, c.3459_3462delCAAT, c.4805delTGAT), 2 insertions (c.637dupA, c.4960_4988dup) and 1 splice site mutation (c.3496+1G>A). With a mutation detection rate of 65% in this selected group of patients, which is within the expected range, our method proved to be an efficient tool for detection of known and new point mutations in the NF1 gene. Currently, in order to improve overall mutation detection rate, as a second step patients without identifiable point mutation are being screened for large deletions and the results will be presented.

P1002. Psychometrics and genetics of nicotine dependence

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Nicotine dependence is a complex behaviour for which a substantial genetic contribution is assumed. Several candidate genes for this genetic contribution have been suggested involving neuroreceptors and metabolic enzymes of nicotine degradation. In order to elucidate their impact in correlation with anamnestic data (Fagerström Score, number of cigarettes per day, sociodemographic data) and abstinence rates, we recruited 202 healthy volunteers who wanted to join a supervised abstinence program.

Using psychometric scales, we identified four subgroups of regular smokers - depressive, hyperactive, highly dependent and “nonclinical” smokers with sample proportions of 16%, 21%, 34%, and 29%, respectively. Cluster membership significantly predicted smoking outcomes 6 and 12 months following a combined pharmocological and behavioural intervention for smoking cessation.

For genetic analyses, this study group has been extended to 272 samples and genotyped for several informative single nucleotide polymorphisms (SNPs) in “nicotine”-related pathways such as (a) the dopaminergic system (ANKK1, DRD3, DRD4, MAOA, COMT, SLC6A3), (b) the serotoninergic system (SLC6A4, HTR2A), (c) the nicotinergic and the cytochrome system (CHRNA4, CHRNA7. CHRNB2, CYP2A6, CYP2D6). Genotyping systems have been implemented onto the new Roche LightCycler480 instrument using the 384-well format and the HybProbe format.

Although univariate association analyses did not harvest an isolated genetic determinant of heavy nicotine dependence, we predicted the number of cigarettes per day based upon genotypes for ANKK1, DRD3, and COMT (categorical regression analysis). A multivariate analysis aiming to identify possible links between the psychometric groups and the genotype data is under way.

P1003. Evidence of exclusion of NKX2-5 gene for atrial septal defect in a consanguineous Tunisian family

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Atrial septal defect (ASD) is an autosomal dominant disease characterized by left-to-right shunting and increased right ventricular output. Approximately 5-10% of congenital heart diseases (CHD) are due to ASD which is one of the most frequent CHD found in human adults.

The gene responsible for ASD has been mapped to chromosome 5q35 by genetic analysis in large pedigrees. This gene encodes the transcription factor NKX2-5 important for regulation of septation during cardiac morphogenesis. *Nkx2.5,* was among the first evidence of a genetic cause for congenital heart disease and mutations were initially found in pedigrees with autosomal dominant transmission of atrial septal defect.

We report here clinical and molecular investigation of a Tunisian consanguineous family with 4 affected members: the clinical features of 2 patients among the 4 affected individual are characterized by ASD with prolonged PR interval whereas the 2 others presented only a prolonged PR interval. Pedigree analysis is consist with an autosomal dominant inheritance of the disease.Genotyping of the ASD family with 2 microsatellite markers D5S394 and D5S2439 flanking *Nkx2.5* gene and Linkage analysis showed exclusion of linkage between the gene responsible for ASD in this family and *Nkx2.5* gene, thus confirming genetic heterogeneity of this phenotype.

P1004. Large family with autosomal recessive mental retardation not linked to 3 known loci.

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Mental retardation (MR) affects approximately 1-3% of the general population. A defining feature of MR is an intelligence quotient (IQ) of less than 70. The aetiologies of MR are diverse and include chromosomal anomalies, recognisable malformation syndromes, monogenic syndromes, structural brain abnormalities, and environmental factors. Genetic aetiologies are found in approximately two thirds of cases. Mental retardation that affects individuals without major physical stigmata, chromosomal anomalies, or fragile X syndrome is called non-syndromic or non-specific mental retardation (NSMR).

Searching for the genes responsible for NSMR is difficult owing to heterogeneity and the absence of clinical criteria for grouping the NSMR families for linkage analysis. We evaluated a large Tunisian family with four consanguinous branches including 14 affected and 22 non affected individuals They had severe mental retardation (IQ<50), epilepsy, absence of speech. Investigation revealed normal caryotype and FMR1 gene. Brain scan and MRI of 2 affected individuals were normal. Twelve microsatellite markers on chromosomes 3p25 ; 4q24-q25 and 19p13.12-p13.2 were amplified by polymerase chain reaction (PCR).

Linkage analysis was performed using the program linkage (version 5.0). The results of two point linkage analysis with these markers show no linkage to the 3 known loci 3p25-pter, 4q24-q25 and 19p13.12-p13.2 and confirm exclusion to those loci.

A genome scan is started for mapping a new gene for non specific autosomal recessive mental retardation.

P1005. Prevalence of GJB2, GJB6 and A1555G mutations in the Italian population

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Mutations in the connexin 26 encoding gene (GJB2) have been described as major cause of non-syndromic hearing loss (NSHL). Deletions involving connexin 30 (GJB6) were also associated with NSHL; A1555G mutation in mitochondrial (mt)DNA has been shown to predispose to aminoglycoside ototoxicity.

Aim of our study was to evaluate the prevalence of these molecular defects in the Italian population. 133 patients with mild to profound, familiar or apparently sporadic NSHL were studied. 513 unaffected controls were also screened to evaluate the carrier frequency. Mutation screening by DHPLC and sequencing for GJB2, multiplex PCR for 309kb and 232kb deletions in GJB6 and restriction analysis for A1555G mutation were performed.

38 patients (28,6%) showed molecular variants of GJB2. In addition to known mutations two novel defects (G109W and 153delT) were found in compound heterozygotes with 35delG. Six novel variants (G>C -3277, IVS1-6T>C, IVS1-2A>C, Y158Y, K221N, N62N) were also detected. One patient (0.8%) carried the 309kb GJB6 deletion and one showed homoplasmic A1555G mtDNA mutation.

61 controls (12%) showed molecular variants of GJB2; 25 (1:20) were carriers of disease-causing mutations. Four novel variants (IVS1-6T>C, IVS1-2A>C, A/G at -8, D159N) were also detected.

In conclusion, GJB2 mutations resulted responsible for almost one third of the NSHL in our patients and a high prevalence of mutation carriers was shown in our population. The low frequency of GJB6 deletions and A1555G mtDNA mutation suggests that the occurrence of these defects is restricted to specific populations.

P1006. Mutational analysis of the *PTPN11* gene in 12 patients with Noonan or Noonan-like phenotype

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Noonan syndrome (NS) (OMIM #163950) is an autosomal dominant disorder consisting in Turner-like phenotype (triangular face, downslanting palpebral fissures, ptosis and short stature), congenital heart disease (most commonly pulmonary stenosis) and skeletal anomalies. Mild mental retardation or developmental delay is common. Its incidence ranges from 1/1000 to 1/2500 live births. Penetrance is

incomplete and more than 1/3 of the cases are sporadic. In 2001, mutations of the PTPN 11 gene in chromosome 12q24 were found in patients with NS. Subsequent studies reported that mutations in this gene account for approximately 50% of the cases.
We present the results of the molecular analysis of the PTPN11 gene in 12 spanish patients with clinical criteria of Noonan syndrome or Noonan-like phenotype. Clinical findings were classified and evaluated following the scoring system proposed by van der Burgt in 1984. Methodologically we performed the PCR-based analysis and sequencing reported by Tartaglia et al. in 2002 for exons 2, 3, 4, 7, 8, 12 and 13, the ones that more likely host mutations.
Two missense mutations were found in two non-related patients with typical NS phenotype. The first one was a T184G (Y62D) in exon 3 in a 10 moth old male with pulmonic stenosis and scalp defects, the second was a G1507C (G503R) in exon 13 in a 11-year old girl without heart defect. No mutation was found in any of the 10 remaining patients, although we are analyzing the remaining exons in the patients with the more striking phenotypes.

P1007. Norrie disease

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Norrie disease is a rare X-inked recessive (Xp11.2-11.3) condition characterized by congenital blindness in males due to degenerative and proliferative processes in the neuroretina . Retinal dysgenesis occurs early during embryogenesis and is often associated with microphthalmia. The most prominent feature at birth is an intra-ocular mass which can be misdiagnosed for retinoblastoma and ultimately leads to shrinkage of the eye globe. The main histopathological findings are rosettes of immature retinal cells embedded in a vascular connective tissue of hyperplastic primary vitreous. Norrie disease is a rare disorder, its exact incidence is unknown. It is not associated with any specific racial or ethnic group.
Mutations in the NDP cause Norrie disease. In affected males, mutations in the *NDP* gene are associated with a spectrum of retinal findings ranging from Norrie disease (ND) to X-linked familial exudative vitreoretinopathy (FEVR), including some cases of persistent hyperplastic primary vitreous (PHPV), Coats disease, and advanced retinopathy of prematurity (ROP).These phenotypes appear to be a continuum of retinal findings with considerable overlap.The ocular findings that permit a presumptive diagnosis of an *NDP*-related retinopathy include the following:
Bilateral, often symmetric, involvement of the eyes
Normal-sized eyes, with normal anterior chambers and clear lenses at birth (usually)
Vitreous abnormalities (hemorrhage, membranes, detachment, and/or vitreoretinal attachments)
Presence of fibrous and vascular retinal changes at birth with progressive changes through childhood or adolescence .

P1008. Genetic background of perceiving odours: a genome-wide screen with Finnish families

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We studied the genetic component of perceiving odours in a novel way by utilizing psychophysical smell testing of human subjects combined with genetic linkage analysis. A total of 146 Finns (100 females, 46 males, aged 18-78 years, mean 49 ± 15 years) from 26 extended families were genome-scanned (using 360 microsatellite markers) and the sense of smell was tested using The Brief Smell Identification Test™ which contained identification (multiple-choice with 4 alternatives) of 12 odours (cinnamon, turpentine, lemon, smoke, chocolate, rose, paint thinner, banana, pineapple, gasoline, soap, onion). Ratings of intensity and pleasantness of each odour (5-point category scales) were added to the test for this study, and the data were standardized before genetic analyses. Heritability estimates were calculated and variance components linkage analysis made using MERLIN program. Score of right identifications was not found to be heritable, but when odours were analyzed separately, several traits showed moderate heritability. Pleasantness of cinnamon odour (104 subjects were phenotyped for this trait) had the highest heritability of studied traits (h^2 = 61 %) and a significant linkage was found between it and chromosome 4q32.3 (multipoint LOD score 3.01).
In addition, moderate heritability (h2 = 31) and suggestive linkage to chromosome 2p14 (multipoint LOD score 2.55) were found for intensity of paint thinner. The results suggest that the way in which some odours are perceived is genetically modified.

P1009. Association of intronic polymorphisms in the ER-a gene with knee osteoartjritis (KOA)

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Osteoarthritis (OA) is the most prevalent joint disease especially over age 60. Estrogens affect articular cartilage metabolism directly via estrogen receptors (ER) in chondrocytes. The ER-α gene is located on chromosome 6q25-27 consisting of eight exons. To elucidate the possible role of the PvuII (P/p) and XbaI (X/x) polymorphisms in intron 1 of the gene on KOA, a case-control study of a homogeneous Greek population was set up. These two polymorphisms have been associated with bone mineral density (BMD) and osteoporosis in different populations. The osteoarthritic group consisted of 158 patients, 138 women (mean age 68.1 ± 8.2; range 48-92 years) and 20 men (mean age 72.4 ± 5.8; range 62-85 years). All of them suffered from severe KOA, defined by a Kellgren-Lawrence score ≥ 2. The control population consisted of 193 subjects, 137 women (mean age 68 ±10.9; range 44-87 years) and 56 men (mean age 70.2 ± 9; range 46-88 years), who had undergone treatment for injuries and fractures. A significant difference was observed in the frequency distribution of ER-α-XbaI (P<0.0001) between OA patients and controls with allele x exerting a protective role and Xx genotype conferring increased risk compared to xx (OR 0.33; 95%CI 0.13-0.84; P>0.02). A significantly increased odds ratio for KOA was also observed in individuals having haplotype PX (double the risk for knee OA) compared to px (OR 1.86; 95% CI 1.21-2.87; P>0.005) while haplotype pX conferred a six times increased risk compared to px (OR 6.03; 95% CI 1.64-24.2; P>0.007).

P1010. A genome-wide linkage scan for low bone mineral density at the lumbar spine in a single extended family provides suggestive linkage to 1p36.3.

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Peak bone mineral density (BMD), which is under strong genetic control, is a major determinant of risk for osteoporosis. Because of a high degree of genetic heterogeneity, multiple genes influence susceptibility to low peak BMD. Yet, it is believed that single major genes play a crucial role in familial inheritance of low BMD. To search for a genetic locus that explains a major part of the variation in susceptibility of low peak BMD we performed a genome-wide screen using 380 microsatellite markers in a single extended family (n=31) with high prevalence of low spinal BMD (Z(BMD)≤-2) and characterised by a young onset. The pattern of inheritance of the low BMD trait in this family is suggestive for autosomal dominance. A two-point linkage analysis was performed making use of quantitative-trait and affected/unaffected-trait parametric models. Next to this a non-parametric linkage analysis (NPL analysis) was carried out.
The highest LOD-score (2.83) obtained in two-point analysis, when an affected/unaffected-trait model was used, was at 1p36.3 (D1S468). The chromosomal region at 1p36 has been previously implicated in several other linkage studies of BMD. Additional microsatellite analysis revealed critical recombination events restricting the candidate region to 1.6 Mb and about 19 genes. Sequencing analysis of the coding region of one of the candidate genes, WDR8, shown to be expressed

during endochondrial ossification, revealed no mutations or disease-associated polymorphisms. Mutation screening for other candidate genes is in progress. In conclusion, our results are confirming earlier findings that there may be genetic determinants for BMD on 1p36.

P1011. Linkage of a Familial Form of Osteoporosis to Chromosome 5q34

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A number of chromosomal loci have been identified and confirmed to a quantitative trait locus (QTL) such as bone mineral density (BMD) by whole genome scans and by scanning candidate regions. In this study a genomewide linkage scan was performed in an extended family with a familial form of osteoporosis.

400 microsatellite markers spread across the 22 autosomes and X chromosome were analysed in 9 family members, eight females and one male. The phenotype was defined by lumbar and femoral z-scores calculated after measurement of bone mineral density (BMD) by DEXA. Multipoint parametric and non-parametric linkage analyses were performed by EasyLinkage v4.0 using GENEHUNTER v2.1, assuming dominant and recessive modes of inheritance with variable penetrance.

Evidence of linkage was observed to a marker at 5q34 where a non-parametric LOD score (NPL) of 7.17 was observed. A parametric LOD score of 2.78 (p=0.0005; info=0.90) for this region was obtained for the recessive mode of inheritance with 80% penetrance and a phenocopy rate of 1%. The disease allele frequency was assumed to be 0.001 (0.10%). Suggestive linkage (LOD scores >1.5 and/or p<0.01) was observed at a number of other chromosomal loci including 6q22, 9q21, 11p13, 13q33 and 17q23.

These results suggest that a major gene might be involved in the onset of this familial form of osteoporosis. It is also evident that other genes might also be involved. Identification of these genes and their roles played in the pathophysiology of disease is important for both prevention and treatment of osteoporosis.

P1012. Association study of haplotype H2 of P2RY12 gene with coronary artery disease and myocardial infarction

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BACKGROUND: Platelet activation and aggregation are key elements in coronary atherosclerosis. Platelet receptor P2Y12 stimulated by adenosin diphopsaphate (ADP) modulates affinity of glycoprotein IIb/IIIa for fibrinogen, resulting in fibrinogen binding and platelet aggregation.

A haplotype of the P2RY12 gene (H2 haplotype) was found to be associated with maximal aggregation response to ADP in the general population and to increase risk of peripheral arterial disease.

Aim of this study was to test the association of the H2 haplotype with coronary atherosclerosis and myocardial infarction.

METHODS AND RESULTS: We studied 1422 unrelated consecutive Italian patients of both sexes. They were diagnosed by coronary angiography as affected by coronary artery disease (CAD or cases, n=1033) or not (CAD-free controls, n=389). In the CAD group 616 patients had a history of myocardial infarction (MI). All patients were genotyped for the H1/H2 haplotype tag SNP of the P2RY12 gene by melting curve analysis of fluorescent real time PCR.

We observed a significant difference in genotype distribution between CAD and CAD-free groups (p=0.024). H2 haplotype carriers were more frequent in the CAD group than in the CAD-free group (p=0.041, OR=1.35, CI=1.00-1.83). No significant difference in H2 haplotype frequency was observed between MI and MI-free groups within CAD population.

CONCLUSIONS: These results suggest an association of the H2 haplotype of the P2RY12 gene with coronary artery disease. No significant association was observed between the H2 haplotype and history of myocardial infarction in the studied population.

P1013. A locus for an autosomal dominant paroxysmal abdominal pain maps to 8q12.3-13.3

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We identified an extended family in Antioquia (Colombia) with multiple individuals presenting abrupt crises of severe abdominal pain. These episodes occur from infancy and are characterized by very intense spasmodic pain, predominantly in the abdomen but occasionally generalised. Episodes are associated with stiffness of abdominal wall, profuse sweating but not loss of consciousness. One episode can last for 30 to 60 min, the pain gradually becoming more severe as the crisis progresses. We carried out a whole genome linkage scan using ~550 microsatellite markers (deCode Genetics Genotyping Service) with an average spacing of 6cM. Parametric linkage analysis was carried out using a model of autosomal dominant inheritance with high penetrance (0.985). Two-point LOD scores ≥ 2, suggesting genetic linkage, were found on chromosomes 2p, 3p, 8q, 9q, and 13q. A single two-point LOD score ≥ 3 was obtained on chromosome 8q12.3, for marker D8S512 ($Z=4.18$ at $\theta=0$). Multipoint LOD score analysis of chromosome 8 yielded a maximum LOD score of $Z = 4.42$ in the interval D8S512-D8S279, notably in the same region that had also shown the most significant LOD score in the two-point analysis. We therefore conclude that we identified a region on chromosome 8q12.3-13.3 that harbours a predisposing gene for familial paroxysmal abdominal pain. Fine-mapping and sequencing of candidate genes in the region is underway.

P1014. High frequency of SDHB mutations in a series of head and neck paraganglioma from Belgium

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Mutations of SDH genes encoding subunits of complex II of the mitochondrial respiratory chain are involved in the pathogenesis of paraganglioma (PG) and pheochromocytoma. While SDHD is more frequently involved in the pathogenesis of head and neck PG, SDHB mutations are mainly associated with malignant and/or extra-adrenal pheochromocytoma. In the aim to looking for the nature and frequency of SDH mutations as well as for possible genotype-phenotype correlations in head and neck PG from Belgium, we recruited all patients with head and neck PG seen in the main Academic Centers in Belgium from May 2003 to December 2005. Screening of the coding parts of SDHD and SDHB was performed by SSCP and heteroduplex analysis, followed by sequencing whenever a shift was observed. Six different SDHD mutations were found in 7 different patients including 5 familial cases and 2 apparently sporadic cases. Four of the mutations were not described previously. Furthermore, 2 different SDHB mutations were found in 4 unrelated patients with apparently sporadic PG. One of them, found in 3 of the 4 subjects, had been already described in a family with malignant pheochromocytoma. (Young et al., JCEM 2002; 87: 4101-4105). Surprisingly, in this Belgian series, SDHB mutations were almost twice as frequent as SDHD mutations (13 vs. 7%) in sporadic head and neck PG without evidence of dissemination, mainly due to a single mutation previously associated with familial metastatic pheochromocytoma (alexandre.persu@nefr.ucl.ac.be).

P1015. COMT and MTHFR genes analysis in patients with Parkinson's disease from Bashkortostan

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The etiology of idiopathic Parkinson's disease (PD) is considered to be multifactorial with both genetic and environmental factors

modifying the disease expression. Recent studies suggest that polymorphism in cotechol-O-methyltransferase (COMT) and methylentetrahidrofolatreductase (MTHFR) might influence the risk of PD. The aim of the study was to evaluate effects of Val158Met and C677T genetic polymorphisms on different forms and severity of PD. Allele and genotype frequencies distribution didn't show significant differences between PD patients and healthy individuals both in common sample of patients and controls ($\chi2=0.58$; P=0.74) and in ethnically differing groups. After dividing PD patients into groups, taking into consideration the disease forms, we revealed that genotype L/H was the genetic marker of decreased risk in patients with akinetico-rigid-trembling form of PD ($\chi2=3.81$; P=0.05; OR=0.59; CI=0.34-1.00), whereas L/L genotype was the genetic marker of the increased risk for patients with akinetico-trembling-rigid form ($\chi2=5.23$; P<0.05; OR=5.30; CI=1.20-22.20). After dividing patients into groups, based on the disease manifestation, we detected that L/L genotype was a risk factor in patient with the disease onset from 40 till 60 years old ($\chi2=5.23$; P<0.05; OR=2.59; CI=1.12-5.96), while L/H genotype was the genetic marker of the decreased risk marker for the same group ($\chi2=4.12$; P<0.05; OR=0.56; CI=0.32-0.98). MTHFR C677T polymorphism revealed no statistically significant differences between common sample of patients and controls as well as in subdivided group of patients taking into account their ethnic origin, PD forms, disease manifestation. So, our results didn't support the hypothesis of pathogenic role of homocysteine in Parkinson's disease development.

P1016. Screening for DJ-1 mutations in early onset Parkinson's disease (PD)

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Mutations in DJ-1 gene cause an autosomal recessive juvenile onset form of PD. In this study we analysed the DJ-1 gene in 40 sporadic patients with early onset PD (≤45 years) and 100 appropriate controls, originated from Southern Italy. These patients were negative for Parkin and PINK1 mutations.

DNA was extracted from peripheral blood using standard protocols and each exon was amplified and sequenced. Absolute quantification was performed by real time PCR 7900 HT-SDS, using TaqMan probes for exons 2,3 and 7. For exons 1,4,5 and 6 we set up a Sybr Green assay.

In a patient we detected an heterozygous nucleotide change (C→G) in intron 1 (nucleotide 10703, ref. AL034417) and a single heterozygous insertion mutation (IVS4+3insA) in intron 4 splice site. These novel variants were not found in 100 control subjects. In the same patient genomic rearrangements were excluded by absolute quantification in real time PCR.

The relevant effect of DJ-1 is its putative role in neuroprotection. Cells lacking DJ-1 have been shown to be more sensitive to cellular stressors.

The IVS4+3insA mutation lies in a conserved sequence of the invariant AG splice acceptor site of intron 4. We hypothesized that this variant could have consequences on DJ-1 RNA, leading to an aberrant transcript. Moreover, the detected intronic change is potentially functional because it is localized 159 bp after the transcriptional activation site and approximately 42 bp downstream of an SP1 binding site. Synergic action of both heterozygous variants could account for clinical features of the examined patient.

P1017. Evidence for novel loci for late-onset Parkinson's disease in a genetic isolate from The Netherlands

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We studied patients with idiopathic Parkinson's disease (PD) from an isolated population in the Netherlands aiming to map gene(s) involved in PD susceptibility. A total of 109 parkinsonism patients were independently ascertained, of whom 62 presented late-onset, idiopathic PD. Genealogical research showed that 45 index cases with idiopathic PD were linked to a common ancestor, indicating familiar clustering among the patients. This strong familial clustering was highly significant (p=0.005) when compared to random controls from the same population. We performed a genome wide scan using 382 polymorphic markers in 44 distantly related PD patients plus 112 unaffected first-degree relatives and spouses.

Our genome wide association analysis (DISLAMB) revealed evidence of association at a nominal p-value <0.01 for markers D2S2333, D4S405, D9S158, D13S153. Other regions on chromosomes 3p, 4q, 14q, 17p and 17q were found at a significance level of p<0.05. In a follow-up study we investigated all the positive regions using a denser marker set and a larger sample (total of 630 individuals including all late-onset PD patients). The strongest evidence for association remained for the 9q and 14q region. A significant association was found for marker D9S1838 (OR=2.0, 95% CI 1.1-3.5, p=0.014), D9S312 (OR=2.0 95% CI 1.0-4.2, p=0.047) and D14S65 (OR=3.2, 95% CI 1.7-6.1, p<0.001), D14S265 (OR=2.2, 95% CI 1.2-3.9, p=0.006). Moreover, a common haplotype with excess of sharing among late-onset PD cases was observed on both regions. Our results suggest the existence of two loci influencing PD susceptibility on chromosome 9q and 14q.

P1018. Screening for PINK1 mutations in patients with early- and late-onset Parkinson's disease

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Mutations in the PTEN-induced kinase (PINK1) gene located within the PARK6 locus on chromosome 1p35-p36 were recently identified in patients with recessive early-onset Parkinson's disease. In this study, we performed a mutational analysis of PINK1 in 12 families, 4 with early-onset and 8 with late-onset parkinsonism, and in 23 sporadic patients with early-onset (age of onset ≤ 45 years of age) Parkinson's disease. The patients originated from Southern Italy. Mutations in parkin and DJ-1 were excluded previously in all patients.

DNA was extracted from peripheral blood according to standard protocols. The 8 exons of the gene with their exon-intron boundaries were amplified using polymerase chain reaction (PCR). Obtained fragments were sequenced on ABI3100 automated DNA sequencer (Perkin Elmer- Applied Biosystems).

A novel homozygous deletion (889delG) was detected in the affected members of a consanguineous family with early-onset parkinsonism and was absent in 100 normal chromosomes. We also identified several exonic and intronic polymorphic variants, most of whom already described.

Sequence analysis of the PINK1 gene led to the identification of a homozygous deletion in exon 4 in two sibs from a consanguineous family with early-onset parkinsonism. The deletion produces a premature stop codon and a protein lacking in most of the kinase catalytic domain. The screening of the remaining patients revealed presence of several polymorphic variants. These findings confirm that recessive mutations in PINK1 cause early-onset parkinsonism, although PARK6 is not a common locus for PD in our population.

P1019. Association of glutathione-S-transferase genes polymorphisms with placental insufficiency

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Placental insufficiency (PLI) is a key problem of modern obstetrics. Its etiology is variable and mostly unclear. Interactions between noxious environmental factors and unfavorable genetic background are suspected to play a decisive role in PLI origin. Personal susceptibility to adverse effects of environmental factors, including chemical exposure, mainly depends on individual peculiarities of genetic polymorphism. Glutathione-S-transferase genes are responsible for xenobiotics conjugating enzymes of Phase II detoxification system and have

been implicated as risk factors for PLI. The genetic polymorphisms of three genes GSTM1, GSTT1 and GSTT1 were studied by PCR/RFLP analysis in placentas with and without PLI. The frequency of GSTM1 0/0 genotype in placentas with intrauterine growth restriction (IUGR) was significantly higher if compared with this one in the control group (61.6 % and 35.3 % respectively, p<0.05). The frequency of D/-- genotype (D= B or C alleles) of the GSTP1 gene was 3 times higher in group with IUGR, than in group without PLI (p<0.01). It was shown that smoking in family in a combination of functionally weakened genotype of GSTP1 gene in placenta could be a risk factor of developing IUGR. Concordance of GSTT1 0/0, GSTT1 + and GSTP1 D/-- genotypes was found in 31% in group with IUGR in comparison with controls (8.7%) (OR 4.6, CI: 1.16-18.1). Thus the polymorphism of glutathione-S-transferase genes might be a risk factor of developing IUGR.

P1020. Association of some vascular genetic markers with preeclampsia

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Preeclampsia (PE) is a hypertensive disorder specific to pregnancy and is a major cause of maternal and neonatal death and morbidity worldwide.

The methylenetetrahydrofolate reductase (*MTHFR*), endothelial nitric oxide synthase (*eNOS*) and tissue plasminogen activator (*TPA*) genes may also play a role in the pathogenesis of PE. These loci are located on different chromosomes and encode products involved into various metabolic pathways leading to PE. The specific genes involved may depend, at least in part, on the characteristics of the population being studied (ethnicity, severity of the disease, maternal age, or gestational age at onset).

We studied the *eNOS VNTR* polymorphism in 4 intron, *TPA Alu* repeat *I/D* polymorphism in 8 intron and *MTHFR C677T* polymorphism in women with PE from Bashkortostan (Russia).

DNA from 132 preeclamptic pregnant women and 172 healthy control pregnant women were genotyped for polymorphisms using PCR technique and subsequent enzyme digestion.The frequency distributions of the genotypes of these three polymorphic marker loci of genes were similar in the case and control groups. We found no differences in the prevalence of genetic risk factors with PE compared with controls. These polymorphisms are unlikely to be risk factors for PE in our sample.

P1021. Genetic polymorphism of the HLA-G gene in severe preeclampsia

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Preeclampsia is a multifactorial disease of pregnancy. The large number of epidemiologic studies supports its heritable nature. Immunological maladaptation theory is one of the leading hypotheses of preeclampsia origin. According to it the maternal immune system does not adequately adapt to the semi-allogenic fetus. Polymorphism in the 3'-UTR of the HLA-G gene has been found to be associated with differences in HLA-G expression and it might be involved in preeclampsia pathogenesis.

The patients with severe preeclampsia (n=47), women without any gynecologic complications and background disorders (controls, n=46) and unrelated individuals from North-West region of Russia (population, n=109) were included in this study. A -14bp/+14bp polymorphism in 3'-UTR region of the HLA-G gene was studied by PCR assay.

The distribution of HLA-G genotypes was in accordance with the Hardy-Weinberg Equilibrium (p>0.05).

Although the frequency of +14/+14 genotype was 2-fold higher in patients (20.9%) compared to the control group (10.9%), the difference was not statistically significant (p>0.05). However, the frequency of +14/+14 genotype of the HLA-G gene was significantly higher (28.6%, p<0.05) in patients with pure form of severe preeclampsia (without background disorders, n=28) and controls.In conclusion we should point out that despite of rather small samples, the carriers of +14/+14 genotype of the HLA-G gene have significantly higher risk of preeclampsia as compared to women, with one or two -14 alleles. Thus there is already substantial evidence supporting a role for HLA-G polymorphism in preeclampsia.

P1022. Polymorphisms in the Endothelial cell protein C receptor (EPCR) gene as potential risk factors for pregnancy loss in the Israeli population

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The endothelial cell protein C receptor (EPCR) and its soluble form (sEPCR) play a role in venous thromboembolism. To identify new thrombophilic risk factors of unexplained pregnancy loss (PL), we studied the EPCR coding gene sequences, known single nucleotide polymorphisms (SNPs) and sEPCR levels in women with PL. Three SNPs in the EPCR gene were evaluated in 249 Israeli women with PL and 254 women without pregnancy complications. The C allele of SNP 1651 C>G in the 5' untranslated region (5'UTR) was more common in women with PL compared with controls (51% vs. 38%, OR=1.72, P=0.0003). Genotype 1651 CC was more frequently observed in women with PL compared with controls only among Arabs (41% vs. 20%, OR=2.74, P=0.02) but not in Jews (P=0.1). Genotype 7014 CG (at the 3'UTR) was more common in controls than in women with PL (52% vs. 42%, P=0.02) in both ethnicities, and genotype 7014 GG prevailed in Arab PL women compared with controls (53% vs. 35%, OR=2.08, P=0.02). Haplotypes C-A-G and C-A-C (SNPs: 1653, 6936 & 7014 respectively) were more frequent in the PL group (OR=1.95, P=0.005 and OR=1.96, P=0.022, respectively). Genotypes 6936 AG and 1651 GC were associated with high levels of sEPCR both in women with PL and in controls, however, no differences were found in prevalence of these genotypes and plasmatic sEPCR levels between PL and controls.

Conclusion: Genotypes 1651 CC, 7014 CG and GG in the EPCR gene may serve as new thrombophilic risk factors of PL in certain ethnic populations.

P1023. The human X chromosome in the etiology of Premature Ovarian Failure (POF)

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Premature Ovarian Failure (POF) is a disorder characterized by lack of ovulation and elevated gonadotropin level before 40 years of age. POF has a frequency of about 1% among females and has become a relevant cause of female infertility. A genetic component of the disorder is demonstrated by numerous familial cases and by the frequent observation of X chromosome rearrangements that led to the definition of a POF critical region in Xq.

Characterization of the POF critical region by analysis of a large panel of X chromosome rearrangements showed that most of the breakpoints mapped to a 16 Mb gene poor region corresponding to Xq21. In this region only 3 genes resulted interrupted by the POF associated breakpoints but their role in the pathogenesis remains unclear as extensive mutation analysis on a cohort of italian POF patients failed to demonstrate any causative variants. Recent results from association studies suggested however that one of these genes may contribute to POF as a susceptibility factor.

Accordingly, expression analysis of genes surrounding X;autosome balanced translocation breakpoints in Xq did not reveal ovary-specific genes while analysis of the autosomal regions in all instances showed genes with specific expression in mouse ovaries, thus making them candidate genes for POF. Investigation of the effect of the breakpoints on chromatin organization of X chromosome and autosomal regions, showed specific alterations of chromatin modifications only at the promoters of autosomal genes translocated to the X and indicated a role of the critical region on ovarian gene expression.

P1024. The analysis of association of polymorphisms in HLA-C, HCR and MTHFR genes with development of psoriasis in Bashkortostan

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Psoriasis is a chronic inflammatory skin disorder of unknown etiology likely involves multiple genetic and environmental factors, prevalent in many populations at frequencies ranging from 1 to 3%. Although linkage evidence has been identified at several loci of the human genome, PSORS1 on chromosome 6p21.3 was reported as a major locus for psoriasis susceptibility. *PSORS1* include the candidate genes *HCR* (*C6orf18*), *CDSN*, *PSORS1C1*, *PSORS1C2* and *POU5F1* (*OTF3*). At the PSORS1 locus, the HLA-Cw*0602 allele is the most susceptible allele for psoriasis in various populations.
We have investigated the allele *HLA-Cw*0602* of the *HLA-C* gene, two HCR SNPs (*C325T*, *C477T)* and *C677T* of MTHFR gene for disease association in 237 patients with psoriasis and 237 control subjects from Bashkortostan.
The Cw*0602 allele was associated significantly with psoriasis (OR = 3.34; 95% CI 2.45-4.56, p = 0.0005). The *HCR-325*T* allele frequency was significantly different between the patients with psoriasis and the control subjects (45.6% and 24.1%, respectively, OR = 2.64; 95% CI 1.98-3.52, p = 0.0005). The frequency of the *HCR-477*T* allele in subjects with psoriasis and controls was 7.8% and 1.2%, respectively (OR = 0.62; 95% CI 0.39-0.97, p = 0.039). The analysis of *C677T* of the *MTHFR* gene didn't confirm the association of it with psoriasis: the *MTHFR-677*T* allele occurred with equal frequency 25% in the patients with psoriasis and the control group.
The results suggested that the *C325T*, *C477T* polymorphisms in *HCR* gene and the Cw*0602 allele of the *HLA-C* gene were associated with development of psoriasis in Bashkortostan.

P1025. Whole genome association study for psoriasis using the Quebec LD Map.

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We previously performed a whole genome association study (WGAS) for Crohn's disease using 382 individually genotyped trios from the Quebec Founder Population (QFP) and 165,000 SNPs distributed evenly across the genome at a density of ~1 marker per 17 kb. Utilizing control chromosomes from this study, we determined levels of linkage disequilibrium (LD) across the genome and used this information to construct the Quebec LD map (QLDM). The QLDM contains 81,000 markers, with density adjusted according to variation of LD, resulting in marker spacing of 1 SNP per 10 to 40 kb. This map was used to perform a WGAS for psoriasis using 500 trios from the QFP. We also performed genome-wide conditional analyses using the risk and protective haplotypes from the well established PSORS1 locus. Regions epistatic to or in genetic heterogeneity with PSORS1 were thereby identified. Both the WGAS and the conditional analyses, identified multiple regions with P-values that met the criteria of genome-wide significance. The top candidate regions were further refined by fine mapping. Following fine-mapping, on average the top 21 regions with p values < 10^{-5} (including 8 regions from conditional analyses) spanned approximately 300 kb and contained ~3 genes (excluding regions from PSORS1 locus). Single-gene resolution was obtained for multiple regions. The associated genes are currently being analyzed *in silico* for the construction of GeneMaps which will reveal the underlying genetic etiology of the disease. In addition to our psoriasis study we are also conducting gene discovery programs in more than 20 common diseases.

P1026. Lack of association between the auto-immunity susceptibility allele PTPN22 1858T/620W and systemic sclerosis in the French population.

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The minor allele of the *R620W* missense single nucleotide polymorphism (SNP) (*rs2476601*) in the *PTPN22* (Protein Tyrosine Phosphatase Non-Receptor 22) gene has been reported to be associated with many autoimmune diseases, such as type 1 diabetes, systemic lupus erythematous, rheumatoid arthritis, juvenile idiopathic arthritis, autoimmune thyroiditis or vitiligo. Systemic sclerosis (SSc) is regarded as a tissue connective disease with autoimmune abnormalities.
OBJECTIVE : The aim of our study was to test for association the *PTPN22*620W* allele with SSc in a French Caucasian cohort.
MATERIAL AND METHODS : A case-control study with 121 SSc patients and 103 controls was conducted. Patients and controls were genotyped for the *PTPN22*R620W* SNP.
RESULTS : No association was found between *PTPN22*620W* allele and SSc (7% vs 9.2%, *P* = 0.39). The frequency of the genotypes carrying at least one *620W* allele was similar in both groups (13% vs 17%, *P* = 0.38). The *PTPN22*620W+* genotype was not associated with autoantibody patterns.
CONCLUSION : *PTPN22*R620W* polymorphism is not a susceptibility genetic factor of the SSc in this French Caucasian cohort.

P1027. Protein Tyrosine Phosphatase (*PTPN22)* Gene in a large Tunisian Family affected with Autoimmune Thyroid Diseases

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The *PTPN22* (protein tyrosine phosphatase N22) gene encodes the lymphoid-specific phosphatase which plays a key role as a negative regulator of T-cell activation through its interaction with the negative regulatory kinase C-terminal Scr tyrosine kinase (Csk). We analysed a functional single nucleotide polymorphism in *PTPN22* gene (rs 2476601) and its implication in autoimmune thyroid diseases (AITDs). Our study population concerned a large Tunisian family (Akr family) composed of 10 generations of more than 400 members including 76 patients affected with AITDs and subdivided into 40 patients affected with graves' disease (GD), 13 patients with Hashimoto's thyroiditis (TH) and 23 patients with Atrophic thyroïditis. Genotyping of the *PTPN22* gene 1858 C/T polymorphism was performed by PCR-RFLP technique. Statistical analysis was performed by Family-Based Association Test (FBAT). Our analysis gives a significant association with the C allele under the additive model (χ^2=4.97; p=0.025). Stratifying patients reveals more significant association of C allele in subgroup of patients who carried at least one HLA-DR11 susceptibility allele (χ^2=9.30; p=0.002). These results support the involvement of *PTPN22* gene in the genetic susceptibility to AITDs in the studied families.

P1028. QTL analysis in Campora population : a new locus for BMI on chromosome 1

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Campora is a geographically isolated village of Cilento, South Italy, with a few founders and inbreeding. Recently, Campora population allowed us to identify a new locus strongly linked to hypertension, encouraging the study of this population for the identification of loci involved in other complex traits. A collection of several quantitative traits related to the cardiovascular system have been measured in this population. The

present study is the first linkage analysis of a quantitative trait in this population.
Body-mass index (BMI), an obesity-related trait and a risk factor for cardiovascular diseases, was calculated for 394 adult individuals in the population. These individuals, all related through a 2947-member pedigree spanning 15 generations, were genotyped for 1122 microsatellites on the genome (average marker distance: 3.6 cM, heterozygosity 0.70). To perform the linkage analysis, we broke the very complex pedigree into 92 families including 366 phenotyped individuals, with an optimized use of the maximum partitioning approach to pedigree breaking proposed by Falchi and collaborators (2004).
With the regression-based linkage statistic proposed by Sham and collaborators (2002), we detect a strong linkage on chromosome 1 (position 176.38, LOD= 4.47), robust to the various trait transformations considered. The linkage is also detected with a variance component analysis. Again, the result is robust to trait transformation. This study suggests that linkage study of sub-pedigrees carefully chosen in the Campora population is a powerful strategy to detect new QTL.
Falchi et al (2004) Am J Hum Genet 75:1015
Sham et al (2002) Am J Hum Genet 71:238

P1029. A Quantitative Trait Locus for Human HDL cholesterol on Chromosome 18p.

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Dyslipidemia defined as high total cholesterol, low HDL cholesterol and/or elevated triglyceride levels represents one of the main risk factors for cardiovascular disease. Although many single-gene mutations have been described for rare disorders of lipid metabolism the genetics of dyslipidemias in the general population remains elusive. Many genome-wide screens have been performed in attempt to localize the genomic regions harboring these genes but success has been limited thus far possibly caused by limited statistical power due to limited sample size. To overcome this limitation we combined the primary genotype data from four Finnish genome-wide screens ascertained for type 2 diabetes, familial combined hyperlipidemia and low HDL cholesterol and performed variance-components linkage analysis implemented in MERLIN on the pooled dataset of 1580 individuals from 291 families for lipid traits, fasting serum total cholesterol, HDL cholesterol and triglycerides. Heritability estimates in our sample were 33% for total cholesterol, 49% for HDL cholesterol and 43 % triglycerides using age, sex, BMI, affection status, centre as well as fasting glucose and insulin values as covariates. We observed suggestive multipoint linkage between total cholesterol and chromosome 11q (LOD 2.04) as well as between HDL-C and 18p (LOD 2.92). We used gene-dropping simulations to determine the empirical significance of our findings. According to our 100 simulated genome-wide screens only the HDL cholesterol locus on chromosome 18p remained significant with an empirical p-value of p = 0.01 (95% CI 0-0.05). This region contains several functional candidate genes for HDL cholesterol and warrants further investigation.

P1030. The role of the M235T polymorphism of the angiotensinogen gene on cognitive functions.

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There is evidence that the renin-angiotensin system (RAS) modulates cognitive function, with the angiotensin-converting enzyme gene (ACE), being the most extensively studied. We analyzed association of the angiotensinogen gene (AGT) with cognition in a large family-based cohort from a young genetically isolated population from the Netherlands. Participants were genotyped for the AGT M235T polymorphism. The auditory verbal learning test was used to assess different components of memory (immediate recall, learning, delayed recall, recognition). The Stroop test (card I, II, III) and Trail Making Test (TMT) were used to examine executive function. Regression analysis was performed to study the effect of the AGT M235T polymorphism on the cognitive tests with adjustment for age, sex, inbreeding, education and intelligence. We also evaluated whether hypertension modified the effect of M235T polymorphism.
The 235T allele, which has been related to high angiotensin II levels in plasma in previous studies, was significantly associated with reduced selective attention, as measured by the Stroop test (card I; p value=0.03, card II; p value=0.06), in the hypertension group only. This finding might be explained by an increased risk of cardiovascular complications in the brain of those with hypertension. In contrast, the 235T allele was associated with a better performance in recognition (p value = 0.07), in line with recent studies showing that higher levels of angiotensin II increase performance in memory-related tasks. This effect was not modified by hypertension. Thus, genes encoding RAS components might affect specific cognitive domains through different physiological pathways.

P1031. A novel method for analysis of 5-methylcytosine in DNA by capillary electrophoresis mobility shift

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A simple and completely novel method will be presented for capillary electrophoretic resolution of amplicons derived from methylated and non-methylated gDNA following bisulfite conversion. Growing interest in the role of methylation of genomic DNA in epigenetics is evidenced by the increasing number of publications and awarded grants each year. There are a variety of methods available for methylation analyses, most requiring primers and/or probes specific to either the fully methylated or fully non-methylated sequence, following bisulfite conversion. We now report, for the first time, our discovery of a straightforward analysis of amplicons, following bisulfite-conversion of gDNA, in samples of mixed methylation states. Using a single primer pair for simultaneous amplification of bisulfite-converted methylated and unmethylated gDNA, we found the amplicons can be separated during denaturating gel electrophoresis. The separation efficiency is based on the number of T vs. C (or G vs. A) differences in the unmethylated and methylated sample, and is predictable based on the nucleotide composition of the amplicons. The amplicons are generated using standard PCR protocols, and are directly analyzed after PCR on an ABI PRISM® 3100 Genetic Analyzer. The minimal number of steps, and oligonucleotide primer and probe syntheses that are required for this methylation analysis scheme simplifies the workflow process. The same amplicon sample is suitable for additional analyses, such as bisulfite sequencing, single-base extension, or additional CE analyses. We will also present our PCR conditions for robust generation of amplicons from bisulfite-converted gDNA.

P1032. P53 variants and recurrent spontaneous abortions

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Recent studies indicate that p53 may regulate the response of embryonic cells to diverse environmental stresses. Moreover, it appears that maintaining a fine balance of p53 protein levels within embryonic cells is important for optimal development as both over- and underexpression can lead to different malformations or embryonic lethality. Because altered levels and activity of the p53 protein may be caused by mutations or polymorphisms in both the coding and non-coding regions of the gene we aimed to investigate if variations in p53 are associated with susceptibility for recurrent spontaneous abortion (RSA). We screened 86 Finnish patients (40 couples and 6 women) with unexplained RSA and 96 controls using DHPLC and sequencing. The six intronic and three exonic variations detected have been previously reported and are predicted to be common polymorphisms. When comparing the genotypes and allele frequencies of these variations between patients and controls, the C11992A polymorphism in intron 3, located 29 bp upstream of exon 4, was shown to be significantly

more frequent in the patients than in the controls. Women carrying the rarer A allele have a more than two-fold increased risk of miscarriage (p=0.0193, OR 2.333, CI 1.1305-4.816), indicating that the A allele is associated with RSA. Further studies are, however, necessary to define whether this polymorphism has functional consequences. If the variation has a phenotypic effect, the C11992A variation could be one of the of genetic factors increasing susceptibility to RSA.

P1033. A novel autosomal dominant restless legs syndrome locus maps to chromosome 20p13

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Objective: Restless Legs Syndrome (RLS) is a sensory-motor disorder characterized by a circadian pattern with worsening at night. It affects mostly the legs and it is described by the patient as an urge to move the legs usually associated with unpleasant sensations, but once movement is initiated, the urge to move and the paresthesia fade substantially. It affects up to 10% of the general population, and over 60% of the diagnosed RLS cases are familial. The objective of the present study was to search for genetic factors causing this disorder in the French-Canadian (FC) population. **Methods**: We performed a 10 cM genome-wide scan using a large FC pedigree. For the two-point parametric linkage analysis we used MLINK and for the multipoint parametric linkage analysis we used SIMEWALK2. **Results**: We detected an autosomal dominant locus for RLS mapping to chromosome 20p13, with a maximum multipoint LOD score of 3.87 at marker D20S849. Haplotype analysis revealed a candidate gene interval, flanked by the telomeric end of chromosome 20p (above marker D20S1155) and by a 0.56 cM interval between markers D20S849 and D20S835, that spans 5.2 Mb and contains 84 annotated genes, which are currently under investigation. **Conclusions**: This is the third reported autosomal-dominant locus for RLS and also the first autosomal-dominant RLS locus in the FC population. The present study supports previous work indicating that a fraction of familial RLS cases are caused by major genes acting in a dominant manner.

P1034. Padi4_89*G/A, padi4_90*T/C and padi4_92*G/C SNPs in the gene of the peptidylarginine deiminase citrullinating enzyme type 4 (PADI4) are not associated with rheumatoid arthritis in Hungarian patients

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Rheumatoid arthritis (RA) is a common disease with characteristic autoimmune inflammatory hallmarks. A special feature is the presence of antibodies against anti-citrullinated peptides. The enzyme performing citrullination is coded by the PADI4 (Genbank NM_012387) gene. Recent investigations on Japanese population (Suzuki et al, Nature Gen, 2003;34:395) revealed genetic variations of the gene, which has been proved to be susceptibility factors to the disease. Since there has been no similar data available for Hungarians, we performed analysis using an available DNA pool collected from 200, well characterized patients with RA and 193 apparently healthy age and sex matched controls. The DNA was analysed for three exonic SNPs of the PADI4 gene (namely, padi4_89*G/A, padi4_90*T/C and padi4_92*G/C SNPs; see Nature Gen, rs874881) using PCR/RFLP-methods. In our study, the general distribution of the allelic variants in the healthy population significantly differed from the Japanese population and was similar to that found in the Western European study groups. In our population there was no statistically different accumulation in any of the above SNPs in the patients with RA. These results show that contrary to the Japanese RA patients these SNPs do not mean susceptibility to the development of the disease in the Hungarians.

P1035. New approach for multifactorial disease candidate gene studies based on re-sequencing

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Current single nucleotide polymorphism (SNP) databases focus on alleles that are frequent (>5%) in the general population. In multifactorial diseases, not all susceptibility alleles are expected to be frequent, as shown by CARD15-NOD2 Crohnâ€™s disease allele frequencies which are all < 4% in most Caucasian populations. Prior to candidate gene studies, re-sequencing of patients DNA, for which susceptibility allele frequencies are increased, would therefore be useful to identify putative susceptibility SNP that might be missing in the current databases. In the course of a candidate gene investigation of the multifactorial disease rheumatoid arthritis (RA), our aim was to test patient re-sequencing for the identification of new SNPs.
For 20 RA candidate genes, we re-sequenced DNA from 24 RA French Caucasian patients. Exons, intronic junctions and regulatory elements were sequenced in both directions. SNP identification was performed with an original bioinformatics tool combined to Polyphred software and only SNP observed consistently in both directions at least twice in the sample were selected.
The analysis of the 9600 sequences yielded 151 SNPs, out of which 45 were absent in the public databases. In addition, from the remaining 106 SNPs, 33 were listed in the databases without any frequency information. Altogether, re-sequencing provided useful information for 78 SNPs (52 %).
At the current stage of public databases, re-sequencing of patients DNA for multifactorial disease candidate gene studies appears to be useful, providing new information for 52% of SNPâ€™s identified in this study.

P1036. Association study of PTPN22 gene with Rheumatoid Arthritis in the Tunisian population

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Rheumatoid Arthritis (RA) is the most common systemic autoimmune disease affecting 1% of the adult population worldwide. Genetic and environmental factors have been suggested to play a role in the development of RA. Besides the role of the lymphoid tyrosine phosphatase, encoded by the protein tyrosine phosphatase 22 (PTPN22) gene, in the inhibiton of T cell activation, we analysed a single nucleotide polymorphism (rs 2476601). Our study population concerned 103 Tunisians patients affected with RA and a control group of 117 healthy individuals belonging to the same geographic area. Genotyping of the PTPN22 gene 1858 C/T polymorphism was performed by PCR-RFLP technique. Statistical analysis was performed using the χ^2 (2X2) test and Fisher's exact test. Our analysis showed no statistically significant differences in the PTPN22 gene polymorphism (R620W) genotypes or alleles distribution between RA patients and control individuals (p=0.29 and p=0.282) respectively. These results suggest that PTPN22 gene can exert a minor effect in the development of RA.

P1037. TNFa, IFNA10, IFNA17, and IFN-gamma gene SNPs in Sarcoidosis

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Sarcoidosis is a chronic granulomatous disease of unknown cause, multifactorial etiology characterized by activation of T-lymphocytes and macrophages. To identify genetic factors involved in the pathogenesis of sarcoidosis we investigated SNPs within 4 candidate genes involved in type 1 immune process (TNFa: -1031T>C, -863C>A, -857C>T, -308G>A, -238G>A; IFNA10: 60T>A; IFNA17: 551T>G; IFN-gamma: 874A>T and 875(CA)n repeats. Our case-control study included 89 patients diagnosed according to international guidelines and 215 controls, both of Greek ethnic origin. The five TNFa SNPs were genotyped using the the NanoChip™ Molecular Biology Workstation (Nanogen www.nanogen.com), the IFNA10 and IFNA17 SNPs using PCR/RE digestion and the IFN-gamma using ARMS. The CA repeat number was determined using an automatic sequencer. All our results are in HW equilibrium. By comparing the genotypes and the frequencies of the alleles using chi-square and Fisher's exact test no statistical significance was found. However using the PHASE 2.1 integrated permutation test program in order to get predictions about the haplotypes, statistical significance was found only for the TNFa gene haplotypes.

TNFa predicted haplotypes (PHASE 2.1)			
Haplotypes	Controls: N (freq %)	Sarcoidosis: N (freq%)	Statistical analysis
TCCGG	197(45.5)	65(41.1)	P=0.0019*
TCCAG	4(0.9)	16(10.1)	
TCTGG	109(25.2)	42(26.6)	
CCCGA	0	5(3.2)	
CCTGG	1(0.2)	0	
CACGG	77(17.8)	29(18.3)	
TCCAA	33(7.6)	0	
CCCGG	11(2.5)	1(0.63)	
INFA10-IFNA17 haplotypes			
TG	30(8.2)	16(8.9)	P=0.15
TT	265(72.4)	121(67.9)	
AG	65(17.8)	32(17.9)	
AT	6(1.8)	9(5.1)	
IFN-gamma (CA)n			
11	3(0.9)	1(0.6)	P=0.661
12	159(49.4)	81(46.5)	
13	118(36.5)	73(41.9)	
14	29(9.0)	11(6.3)	
15	12(3.7)	8(4.6)	
16	1(0.3)	0	

P1038. Association study of SNPs in the gene G72 with schizophrenia

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Objective: The *G72* (brain-expressed protein) gene was suggested to interact with D-amino-acid oxidase and to exert an effect on the regulation of *D-serine*, an agonist for the glycine-binding site of the *N-methyl-D-aspartate* - type glutamate receptor. Recently, the *G72* gene was reported to be associated with schizophrenia in the French Canadian and Russian populations (Chumakov et al, 2002). In this study we addressed further the possible role of the *G72* gene in the risk of schizophrenia in population from Bashkortostan (Russia).
We investigated two SNPs (M-15 and M-23) in *G72* gene.
Methods: DNA from 351 patients (131 Russians, 112 Tatars and 108 Bashkirs) with schizophrenia (diagnosed as having ICD-10 (1994) at the age of 15 - 74 and 423 control subjects (115 Russians, 168 Tatars and 140 Bashkirs) were genotyped using PCR - RFLP technique.
Results: Allelic and genotypic frequencies of *M-15* marker did not differ (p>0.05) between patients and control subjects in either ethnic group. A significant differences were observed in *M-23* genotype (χ^2=9.14; p=0.007) and allele (χ^2=9.19; p=0.003) frequencies between patients and control in Tatar origin. The *G72*T* allele was more frequent in Tatar patients compared with controls (χ^2=8.62; p=0.004; df=1; OR=1.75; CI 95% 1.20-2.56).
Conclusions: Our findings suggest that the *G72* polymorphisms may be associated with schizophrenia; however, the effect is influenced by ethnicity.

P1039. Genetic analysis supports a primary abnormality in oligodendrocyte function in schizophrenia

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Abnormal oligodendrocyte function and myelination have been implicated in schizophrenia by a diverse range of experimental approaches including gene expression analysis, neuropathology, and neuroimaging but it unclear whether such abnormalities are of primary aetiological relevance to schizophrenia pathogenesis. It is our aim to resolve these using genetic approaches. *OLIG2* which maps to 21q22.11 and encodes a basic helix-loop-helix transcription factor that is critical for oligodendrocyte development and differentiation. Association analysis of olig2 revealed several associated SNPs associated with schizophrenia in a large UK case-control sample (n individuals ~1,4000; minimum p = 0.0001). In human brain, *OLIG2* expression was significantly correlated with that of *CNP* and *erbB4*, two genes of relevance to oligodendrocyte function for which we have previously reported modest evidence for association with schizophrenia. We sought evidence for genetic interaction between *OLIG2* and the genes showing evidence for correlated expression additional to any main effects. Interaction analysis provided suggested epistatic effects between *OLIG2* and each of *CNP* and *erbB4* even allowing for multiple testing. Our data provide strong support for the hypothesis that oligodendrocyte function is relevant to schizophrenia pathogenesis.

P1040. Association study in the 5q31-32 linkage region for schizophrenia using pooled DNA genotyping and family-based controls

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Schizophrenia (SZ) is a common, severe and disabling disorder that affects around 1% of the general population worldwide. Family, adoption and twin studies have shown conclusively that a genetic component plays an important role in its aetiology. Chromosome 5q31-32 region has been reported as one of the five most consistent linkage regions in the genome as shown from a recent SZ meta-analysis (Lewis et al, 2002). The protocadherine genes localized within this region are also considered to be candidate-genes in the aetiology of schizophrenia due to their role in establishing specific neuronal connections and communication.
We wanted to establish whether the 5q31-32 chromosomal region harbors genes relevant to the pathogenesis of SZ. We are currently saturating the ~30Mb region with microsatellite markers at ~100 kb intervals. We use a database of 27,039 microsatellites validated in a study from Japan on rheumatiod arthritis (Tamiya et al, 2005). This ensured that nearly all microsatellites we genotype are polymorphic.
So far we screened 42 informative microsatellite markers for association with schizophrenia by genotyping pooled DNA from 297 parent-proband trios from Bulgarian origin. Each pooled DNA is genotyped in triplicate. Our cut-off point for following-up pooling with individual genotyping is p<0.1. This level of statistical significance has been found for seven of the markers, four of which are spanning the protocadherin gene clusters. Individual genotyping of positive markers will be performed after the pooling part of the project.

P1041. Identification of an Na_v 1.1 sodium channel (SCN1A) loss-of-function mutation associated with familial simple Febrile Seizures (FS)

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FS affect 5-12% of infants and children up to 6 years of age. Epidemiological studies show that FS are associated with subsequent afebrile and unprovoked seizures in about 7% of patients. Six loci are responsible for autosomal dominant familial FS but no genes were identified: they have been mapped at chromosome 8q13-q21 (FEB1), at 19q (FEB2), at 2q23-24 (FEB3), at 5q14-q15 (FEB4), at 6q22-24 (FEB5) and at 18p11.2 (FEB6). FEB5 has been described as a pure FS because most of the members had a long follow-up period and the development of afebrile seizures was excluded, but pathogenic mutations have not been identified. In the present study we reported a linkage analysis in a southern Italy family, including 23 members of whom 12 individuals expressed a homogeneous phenotype of simple FS. Lod score values were negative for FEB1, FEB2, FEB4, FEB5, FEB6 but showed strong linkage to the FEB3 locus. SCN1A gene lies in this locus, but gene mutations causing simple FS have not been found. Flanking intron primers were used to sequence 26 exons of the SCN1A gene. We found a M145T mutation in a well conserved aminoacid in the first transmembrane segment of domain I, that cosgregated in all affected individuals. Functional studies in mammalian cells demonstrated that the mutation causes a 60% reduction of current density and a 10mV positive shift of the activation curve. Thus, M145T is a loss of function mutant. These results shows that monogenic FS should also be considered a channelopathy.

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P1042. Mutational analysis of SCN2A gene in Italian families with Benign Familial Neonatal-Infantile Seizures (BFNIS)

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Two benign autosomal dominant epilepsy syndromes are well recognized in the first year of life. Benign familial neonatal seizures (BFNS) starts around day 3 and is caused by defects in potassium channel genes KCNQ2 and KCNQ3 in many families. Benign familial infantile seizures (BFIS) begins around 6 months of age but no genes have been definitively identified. Linkage to chromosome 19 and 16 has been reported and a mutation in the ATP1A2 gene was described in one family with both familial hemiplegic migraine and infantile seizures. BFNIS represents an intermediate variant in which seizure onset varied from 2 days to 3.5 months. Recently, mutations were reported in SCN2A, the gene coding for the α2 subunit of the voltage-gated sodium channel in 3 families with BFNIS, (Canadian, Australian and American origins).

In this study we conducted a molecular analysis of SCN2A gene in two BFNIS families from southern Italy (Sicily). The first family with 3 affected individuals over four generations showed seizures onset around 2 months of age; in the second family with 4 affected individuals over four generations seizures start around day 17. After informed consent, DNA was isolated from peripheral blood lymphocytes by standard methods, and was analyzed for mutations in the coding regions of SCN2A (28 exons) by PCR and sequencing. One proband from families was screened.

To date, we have not found any variant in the examined exons of the SCN2A gene (first 25 exons) but the molecular analysis are still in progress.

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P1043. Mutation screening of the *SGCE* gene in patients with Obsessive-Compulsive Disorder and/or Tourette syndrome

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Mutations in the epsilon sarcoglycan gene (*SGCE* gene) are associated with obsessive-compulsive behavior in some families with Myoclonus-Dystonia (M-D). Therefore, we screened the *SGCE* gene for mutations in patients with Obsessive-Compulsive Disorder (OCD) and/or Gilles de la Tourette syndrome (GTS), a genetically related tic-disorder.

We screened the coding region and flanking intronic regions of the *SGCE* gene for mutations in 88 patients with OCD and/or GTS with a positive family history for tics and/or obsessive-compulsive behavior and 5 patients with non-familial OCD. No sequence variants were found in the coding region of the *SGCE* gene and several common polymorphisms were present in the intronic regions of the *SGCE* gene. However, we identified three new sequence variants in the 3'UTR of the *SGCE* gene that were not present in control chromosomes from the general Dutch population. These variants are predicted to be within putative microRNA binding sites and could have a biological function. We are currently investigating additional family members in order to study co-segregation of these variants with the disease in order to decide if functional follow up studies are warranted.

P1044. DNA mutation screening of SLC12A3 gene in a set of patients from the Czech Republic

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For almost three years we have been collecting patients from the Czech Republic suffering from either Gitelman or Bartter syndrome - disorders characterized by hypokalemic metabolic alkalosis with mainly autosomal recessive inheritance. The aim of our study was the characterization of molecular defects; to find causal mutation within selected genes and to look for connection between mutation and severity of disease.

We are presenting here results of mutation screening of SLC12A3 gene - a causative locus for development of renal defect leading to Gitelman syndrome manifestation.

Among a set of 32 patients, 19 were directly diagnosed as having Gitelman syndrome for specific levels of ions in blood and urine. 16 patients of this group have at least one causative mutation in SLC12A3 gene.

We recorded mainly missense mutations (23) but frameshift (3) and splice-site (1) mutations were also found. Novel missense mutation c. 790 G>C, p.Gly264Arg was detected .

No correlation was observed between mutation type and clinical appearance. This result might be influenced by low number of patients diagnosed.

We will describe a case report of one patient who suffered from severe form of arthropathy. It has been shown in history several times that connection exists between Gitelman syndrome hypomagnesemia and chondrocalcinosis. Mutation screening revealed homozygous missense mutation Gly439Ser.

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P1045. Analysis of SMN Mutations in Iranian SMA Patients

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Spinal muscular atrophy is one of the most common autosomal recessive disorders, with a carrier frequency of approximately one in 50.Spinal muscular atrophy can be classified based on age of onset and severity.SMA of all types is associated with homozygous mutations in the survival of motor neurone gene(SMN).Because of high rate of consanguinity in Iranian population, it seems that the incidence of the disease is very high.

During the last five years,mutation detection was performed for 168 families.Among the all of the cases that referred for carrier detection we can found 70 patients with SMAI,11 patients with SMAII and seven patients with SMAIII. Molecular analysis was performed for detection of SMN1 exon 7 deletion.

Chorionic Villus Sampling (CVS) and Amniocentesis were performed for 33 and 10 fetuses, respectively, of which 6, 12, 25 cases were normal, affected and carrier, respectively.

The most common type of SMA in our patients was SMA type I. 90 families with 70 affected cases belong to this group.

The most common clinical findings in patients with SMA typeI was hypotonia with age of onset at birth to 18 month.

In SMA type II we found developmental delay and hypotonia. Also seven patients with SMA type III had age of onset 2.5-30 years were associated with generalized muscle weakness and wasting , tongue fasciculation and decreased DTR.

P1046. Relationship between SCN1A mutations and SMEI

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Mutations in the SCN1A gene are the major cause of GEFS$^+$ which most commonly manifests as febrile seizures (FS), or FS plus. It still remains uncertain if severe myoclonic epilepsy in infancy (SMEI) represents the end of the spectrum within the GEFS$^+$ phenotype, or it should be considered a distinct entity. Here we report three novel SCN1A mutations in three different patients. Two patients had classic SMEI phenotype with early infantile febrile seizures, atypical absences, cognitive impairment, ataxia and drug resistance. The third patient had a milder phenotype and normal neurological development. He had early prolonged FS, afebrile motor seizures and absences, which disappeared with antiepileptic therapy.

The 26 exons of SCN1A gene were individually amplified using primers based on intronic sequences. The purified PCR products were then sequenced and analyzed with an automatic sequencer.

We identified three novel SCN1A mutations. In the first of the two SMEI patients we found a missense mutation, T1289I. His mother and brother carried the same mutation, but they never had any seizure. The second SMEI patient carried a de novo frame shift mutation, 3840insT, that lead to a premature stop codon in the exon 19. The third patient carried a de novo single nucleotide substitution in the invariant AG splice acceptor site of intron 24.

The results of our study reinforce the belief that SMEI probably results from the cumulative effects or interactions of a few or several genes, of which the reported GEFS$^+$ gene is only one player.

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P1047. The SNP technology platform at Uppsala University, Sweden

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The Uppsala WCN SNP technology platform performs SNP genotyping as a service to academic groups in Sweden, the Nordic countries and as a partner in EU-funded projects. Our services include bioinformatics-assisted SNP assay design, SNP assay development, followed by production scale genotyping. We have a staff of five research engineers or laboratory technicians, three biocomputing engineers and a laboratory manager. We use three genotyping systems which are based on primer extension technology. The homogeneous FP-SBE assay is used for genotyping individual SNP, the GenomeLab SNPstream system from Beckman Coulter is used for 12 or 48-plex SNP analysis and for genotyping of 96 to 1536 SNPs we use the Illumina Golden Gate Assay. During 2006 genome-wide SNP analysis using the Illumina Infinium assay will be available as a service. We have developed a relational MySQL database for handling and storing information on samples, SNP-assays and produced genotypes, and for quality assessment of the genotype data. To ensure high quality of management and operation of the SNP platform, we have implemented a quality system according to the European ISO/IEC 17025 standard. Several million quality controlled genotypes have been delivered to over 50 research projects, most of which have been related to human complex diseases. The projects have varied largely in size from one SNP in 77 samples to 3350 SNPs in 1700 samples. The accuracy of our genotyping is 99.6-99.9% (average 99.86%) and typically the success rate is 91-99% (average 95.1%).

P1048. Association of X chromosome located genes of serotonergic system with suicidal behavior

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Differences in suicidal behavior are known between men and women: women attempt suicide four times as often as men, while men complete suicide three times as often as women. Suicidal behavior is associated with a serotonin deficit, and clear sex-specific differences in serotonergic system were demonstrated in a number of studies. Gender specificity in epidemiology and psychopathology of suicidal behavior suggests an X-linked genetic factor. In the present study we performed analysis of some serotonergic system genes located on the X chromosome, namely the *HTR2C*, *MAOA* and *MAOB*, in suicide attempters, separately in men and women. Cases for this study were 283 suicide attempts from 94 men and 189 women. The control group consisted of 261 healthy volunteers: 133 men and 128 women. Three polymorphisms: the *HTR2C Cys23Ser*, *A/G* in intron 13 of the *MAOB* and *EcoRV*-RFLP of the *MAOA* were analyzed using PCR technique. We computed multivariative logistic regressions to examine the joint effects of several variables in predicting the suicidal group. Sex was a significant predictor of suicide group ($p<0.001$). *MAOB*A* allele (coding high activity enzyme) was associated with suicide both in men ($p=0.024$, OR=2.13) and in women ($p=0.016$, OR=1.59). Allele frequencies of the *MAOA* and *HTR2C* did not differ significantly between the control and suicide groups, neither in men, nor in women. *MAOB* ($p=0.003$) and *MAOA* ($p=0.02$) significantly predicted the suicide group independent of the effect of sex. Our findings indicate the contribution of the *MAO* genes to susceptibility for suicidal behavior.

P1049. A new syndromic X-linked mental retardation with severe microcephaly linked to Xp21.1-p11.3

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X-linked mental retardation (XLMR) is classically divided into syndromic (MRXS) and nonsyndromic (MRX) forms. More than 30 genes have been identified in MRXS. We report a large family with five affected males in a pedigree suggesting an X-linked recessive mode of inheritance, presenting with severe mental retardation (5/5 cases), severe microcephaly (5/5 cases), normal stature and facial dysmorphism with hypertelorism and large ears. Carrier females appeared normal. Eye examination (3/5 cases) revealed nystagmus (2/3 cases) or moderate congenital cataract (1/3 case). Cardiac, renal and skeletal investigations were normal. Brain CT scan showed cortical atrophy in 2/2 cases. Chromosome analysis with telomeric studies was normal. Linkage analysis using microsatellite markers spread over the whole X chromosome (10 cM interval) identified a 10 Mb region of

localisation on Xp21.1-p11.3 between markers DXS1110 and DXS1367 with a lodscore of 2.2 (theta = 0) and a common haplotype between affected males and obligate carrier females. Mutation screening in several genes located in this interval and associated with X-linked MR was negative. Microcephaly has already been described in MRXS such as MEHMO (Xp22.13-p21.1), Renpenning (Xp11.23), Borjeson-Forssman-Lehmann (Xq26.3), and Hoyeraal-Hreidarsson (Xq28) syndromes. A normal *PQBP1* gene analysis ruled out Renpenning syndrome in the present family. We therefore think that this family represents a new MRXS with microcephaly mapping to Xp21.1-p11.3.

P1050. A new multifactorial disease gene definitely confirmed by linkage, PTPN22 in rheumatoid arthritis.

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The T allele of the tyrosine phosphatase PTPN22 C1858T/R620W single nucleotide polymorphism has been reported to be associated with rheumatoid arthritis (RA), the most frequent auto-immune disease, in several case-control studies on West European Caucasian populations. The association was observed in the rheumatoid factor positive (RF+) RA subgroup, showing no interaction with HLA-DRB1, the first RA gene. However, the range of allele frequencies in patients overlaps with the range in controls, resulting in a risk of false positive findings. OBJECTIVE : We aimed at confirming the association by linkage, using the transmission disequilibrium test (TDT). MATERIAL AND METHODS : We genotyped 465 West European Caucasian trio families (one RA case and both parents), out of which 345 were RF+. The TDT was followed by an allelic (AFBAC) and genotypic association analysis with odds ratio (OR) and 95% confidence interval (CI). Interaction with HLA-DRB1 was tested using the new modelisation that we recently validated. RESULTS : The TDT demonstrated linkage, showing 61% of transmissions for the 1858T allele (P<0.002), contributed by RF+ RA families (63%, P<0.001), with no linkage in RF- RA families (53%, NS). In keeping with the litterature, the allele frequency increased from 10% in controls to 16% in RF+ RA (P<0.001) and the risk genotype (1858T/620W +) from 18% to 28% (P<0.001 ; OR=1,8 (CI 1,3-2,6)). No interaction was observed with HLA-DRB1. CONCLUSION : This linkage evidence definitely confirms PTPN22 as the second RA gene, implicated in the RF disease heterogeneity, independently from HLA-DRB1.

P1051. Exclusion of candidate genes for a hereditary type of telangiectasia

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Telangiectases are characterised by an abnormal permanent dilatation of end vessels, mainly venules but occasionally also capillaries and arterioles in the subpapillary plexus in the upper dermis. Hereditary haemorrhagic telangiectasia (HHT or Rendu-Osler-Weber disease) is characterized by epistaxis, mucocutaneous telangiectases and visceral arteriovenous malformations (AVMs) caused by mutations in Endoglin (ENG) or activin receptor-like kinase-1 (ALK1). Hereditary benign telangiectasia (HBT) is another, more rare variant. Affected individuals present with cutaneous, punctate, radiating or arborising telangiectases. HBT distinguishes from the more serious hereditary hemorrhagic telangiectasia, by the lack of AVMs and absence of mucosal lesions. These two telangiectasias are inherited as an autosomal dominant disorder with variable penetrance. Another similar hereditary cutaneous phenotype is CM-AVM caused by mutations in RASA1. These patients have more homogeneous, round-to-oval pinkish-red cutaneous lesions, atypical capillary malformations, which are sometimes associated with AVMs.
We report a family with cutaneous telangiectasias with an autosomal dominant pattern of inheritance. The affected individuals develop large, heteregenous telangiectasias, which are not associated with AVMs. This could be a more extensive variant of HBT. We performed a genome-wide linkage analysis using microsatellite markers and SNP genotyping assay with the GeneChip Human Mapping 10K Array. The analysis excludes the three candidate genes, endoglin, ALK1 and RASA1. This provides evidence for existence of a different causative gene for this type of hereditary telangiectasia. (http://www.icp.ucl.ac.be/vikkula) (vikkula@bchm.ucl.ac.be).

P1052. Is M129V of the Prion protein gene (PRNP) associated with mild temporal lobe epilepsy?

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Temporal lobe epilepsy (TLE) is the most frequent form of partial epilepsy in adults. The genetic predisposition appears to be an important causative factor and current evidences suggest that TLE represents a complex phenotype with a polygenic or multifactorial inheritance. Association studies showed that the prion protein gene (PRNP) is highly prevalent in patients with intractable temporal lobe epilepsy and may influence the surgical outcome. We evaluated the genetic contribution of PRNP gene in mild temporal lobe epileptic patients. We analysed the PRNP M129V polymorphism in 289 patients with mild TLE and compared to a neurologically unaffected age and sex matched control group (n=272). Statistical analysis revealed a significantly difference in the distribution at codon 129 of the PRNP gene between sporadic mild TLE patients and healthy controls (p=0.036; OR=1.30; 95% CI=1.01-1.68). Although, there was no statistically significant difference in the genotype distribution within the study groups (p=0.0101), interestingly, we observed that the 129V allele was highly represented only in women with TLE compared to control group (p=0.006, OR=1.632; 95%CI=1.15-2.31). These findings further support the hypothesis that the common methionine/valine polymorphism at codon 129 of the PRNP gene may modify the susceptibility to mild TLE. However, this novel finding warrants replication.

P1053. TRAPS mutations (TNF Receptor gene TNFRSF1A Associated Periodic Syndrome) are frequent in rheumatoid arthritis families but show no evidence for association nor linkage with the disease

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TNFRSF1A mutations cause TRAPS [OMIM*191190]. A recent study suggested that the *R92Q* mutation was associated with chronic polyarthritis. OBJECTIVE : We aimed at searching for *TNFRSF1A* mutations in RA, to be tested for linkage. MATERIAL AND METHODS : the 386 DNA of 100 trio families (one RA case and both parents) and 86 index cases of RA affected sib-pair (ASP) families from the French Caucasian population were investigated by dHPLC (denatured high-performance liquid chromatography) for *TNFRSF1A* mutations in exons 2 to 4. The test for association compared cases and « virtual controls » (derived in the trio families from un-transmitted parental chromosomes). The test for linkage relied on the transmission disequilibrium test (TDT) in trio families and cosegregation in ASP families. RESULTS : Only the *R92Q* mutation was detected, in 2 of the 100 index cases of trio families (including one *de novo* mutation) and 5 (6%) of the index cases of ASP families, but also 6% of the controls, showing no association with the disease. No RA linkage evidence was found : a) out of 7 heterozygous parents in the trio families, only 1 transmission of the mutation was observed ; b) among the 5 RA sibs of the mutated index cases from ASP families, only one carried the mutation. CONCLUSION : This *TNFRSF1A* investigation in RA from the French Caucasian population showed only the *R92Q* mutation, with a frequency of 4%, but no evidence for RA association nor linkage to the disease.

P1054. Anomalous triallelic pattern at FGA locus in pre-transplant sample genotype during chimerism analysis

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Anomalous multibanded signals at a short tandem repeat (STR) loci can be due to a variety of artifacts associated with amplification, detection or contamination. These effects are diagnosed easily by performing a re-analysis, but tri-allelic patterns are sometimes observed at a single locus in a multiplex STR profile. These extra-peaks are not a result of a mixture but are reproducible artifacts of the sample. In a two siblings bone marrow transplantation (BMT) chimerism analysis, the buccal swab recipient's genotype exhibits alleles 22, 23, and 27 at the FGA locus. This tri-allelic banding pattern was restricted to a single locus within a thirteen-locus profile. Before performing BMT analysis, the anomalous profile was studied further and additional tissue samples were collected to establish the genetic basis of the observed pattern. Blood, buccal cells, coutaneus biopsy and plucked hairs, that showing the same multibanded pattern, were investigated to the relative allele proportions. The three alleles profile observed in several tissues excludes the presence of microsatellites instability (MSI) frequently detected in human neoplasia. A systematic examination of this profile showed the unequal signal intensities of the three-allele in all tissue samples examined, suggesting that these mutational changes are due to somatic mosaicism. We concluded that more than one cell lineage was affected and that the size differences between two minor alleles would reflect the magnitude of the mutational change, depending upon the particular stage of tissue development at which the mutational event occur.

P1055. TRPV1 315Ile/Ile genotype is associated to inflammatory pain in a Spanish population.

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Pain stimuli are detected by specialized primary afferent neurons called nociceptors. One noxious stimulus capable of activating these cells is capsaicin which binds to the Transient Receptor Potential Vanilloid 1 (TRPV1) on the peripheral terminals of nociceptive neurons. The observation that capsaicin elicits burning pain and neurogenic inflammation suggested that this receptor could be relevant to nociception.

The TRPV1 gene has two single nucleotide polymorphisms, located in codons 315 (Met315Ile) and 585 (Ile585Val) that produce amino acid substitutions. In an attempt to determine if genotypic variations in TRPV1 gene could modify the susceptibility to suffer either neuropathic or inflammatory pain we have studied the Met315Ile and Ile585Val TRPV1 polymorphisms in patients with each one of these pathologies and in a group of healthy subjects.

A total of 728 subjects, 204 diagnosed as suffering neuropathic pain controlled at the Pain Unit of the University Hospital of Salamanca, 228 diagnosed as suffering inflammatory pain controlled at the Rheumatology Unit of the University Hospital of Salamanca and 296 subjects without a history of pain, matched by age and gender, were included in the study. Consent was granted from the ethical committee of the University Hospital of Salamanca and informed consent was obtained from each subject.

Our results show that variations in the TRPV1 gene do not modify individual susceptibility to neuropathic pain, whereas, in our population the TRPV1 Ile315Ile genotype is associated to higher susceptibility to suffer inflammatory pain.

P1056. An association of TSPY gene copy number with male infertility

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The human TSPY gene family (30- 60 copies) is situated in the MSY region on the Y chromosome. Testis specific expression indicates that the gene plays a role in spermatogenesis. So far, the exact determination of TSPY copies and the interindividual population variability has not been fully described. We applied Refined Quantitative Fluorescent PCR to evaluate the relative quantity of specific TSPY PCR products in comparison to PCR amplicons from single copy AMELY and AMELX gene in 81 stratified infertile men (azoospermic, ≤ 5million sperms/ml, ≥ 5million sperms/ml and AZF deletion) and 40 controls. Our work is supported by IGA MZ CR NR/7821-3. The differences among particular infertile groups were not statistically significant although the AZF deletion category shows the highest rates. We found higher relative values in infertile men in contrast to controls. We evaluated the diagnostic discrimination potential of relative TSPY copies by Receiver Operating Characteristic (ROC) curve analysis. TSPY/AMELY was unambiguously found to be more powerful in the diagnostic separation of both the control samples and the infertile men than TSPY/AMELX. The evaluation of the TSPY copy number could enlarge the diagnostic approached in relation to the genetic cause of male infertility.

P1057. Genetic predisposition to tuberculosis in Siberian populations

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It is well known that tuberculosis (TB) is a complex disease, and polymorphism of genes predisposing to TB plays significant role in the disease development. We studied association between polymorphic variants of five genes of predisposition to tuberculosis: *NRAMP1* (274C/T, 469+14G/C, D543N, 1465-85G/A), *VDR* (F/f, B/b), *IL1B* (3953A1/A2), *IL1RN* (VNTR), *IL12B* (1188A/C) with clinical disease in 304 TB patients and 140 unaffected controls of Russian ethnicity from Tomsk and 238 TB patients and 260 unaffected controls of Tuvinian ethnicity from Tuva Republic.

In comparison between Tuvinians, Russians and other world populations, significant differences of allele frequencies for all studied polymorphisms were found. It confirms the ethnic specificity of polymorphism of genes predisposing to TB. The prevalence of potentially pathological alleles *NRAMP1**543N, *VDR*b*, *IL12B**1188C and *IL1RN**A2 was significantly higher in Tuvinians as compare to other populations. However, in this ethnic group there were no association between these alleles and TB or different clinical forms of the disease. In Russians, association with TB was detected for five of nine polymorphisms investigated (1465-85G/A, 274C/T, 1188A/C, +3953A1/A2, VNTR). Also, a number of associations of the studied polymorphisms with TB clinical signs (blood counts, X-ray data) was found both in Tuvinians and Russians. Thus, in this investigation it was detected that polymorphism of TB susceptibility genes (*NRAMP1*, *VDR*, *IL12B*, *IL1B* and *IL1RN*) in studied Siberian populations (Tuvinians, Russians) is characterized by ethnic specificity with respect to association with TB and its clinical signs.

P1058. Genetic analysis of polymorphic variants of IL1B, IL1RA, NRAMP1, and VDR genes in patients with pulmonary tuberculosis in Republic Bashkortostan (Russia).

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With one third of the world's population infected with *M. tuberculosis*, tuberculosis is the number one cause of death from infectious disease. Analysis of polymorphisms in IL1B (-511C/T), IL1RA (VNTR), NRAMP1 (1729+55del4, D543N) and VDR (FokI, TaqI) genes was carried out in tuberculosis patients (n=195) and healthy individuals (n=190) from

Bashkortostan.
The analysis of the -511C/T polymorphism of IL1B gene demonstrated an increase of the frequency of the genotype IL1B*C/T (75,7%) in the tuberculosis patients compared to the control group (48,9%; P<0.001, OR=3.25). The investigation of VNTR polymorphism in IL1RA gene has shown that the frequency of homozygous IL1RA*1/1 in patients is significantly higher than in the control (73,3% and 43,3%, respectively, P<0.001, OR=3.60). The frequency of heterozygous genotype TGTG/del of NRAMP1 (1729+55del4) was 3 times higher among patients (12,4% and 4,8%, respectively, P<0.001; OR=2.8). We found no significant associations with tuberculosis susceptibility in the case of variations for the NRAMP1 (D543N), VDR (FokI, TaqI). Obtained results thus suggests that variations in IL1B (-511C/T), IL1RA (VNTR), NRAMP1 (1729+55del4) genes probably contributed to development of pulmonary tuberculosis in patients of studied region.

P1059. Screening of the TSC1 and TSC2 genes for Tuberous Sclerosis patients

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We did molecular testing of the TSC1 and TSC2 genes in 90 Tuberous Sclerosis (TS) families, using MLPA, DHPLC, sequencing and microsatellite analysis. A mutation has been identified in 51 cases, analysis is still in progress for 35 cases, and no mutation was found in four cases.
We designed a strategy to screen novel cases, permitting to find more than half of TSC2 mutants and more than 80% of TSC1 mutants in a shorter time than previously.
Two third of the mutations were de novo. TSC2 was more frequently involved than TSC1 (83% versus 17%), specially in those de novo cases (91% versus 9%). In familial cases, TSC1 and TSC2 were almost equally involved.
Only one third of the 51 mutations were previously described. Most of the mutations were truncating mutations. Two large deletions of TSC2, were found.
A better prognosis is ascribed to TSC1 mutations and to familial cases. In one of our families, all the persons with TS were very mildly affected. Unfortunately, clinical severity may vary from person to person in a same family. Nevertheles, half of de novo cases had a favorable outcome.
For cases detected in utero, prognosis was not worse than for cases diagnosed postnataly. We believe it may be better, due to a sharper follow-up.
In summary, even if turn around time can be improved, molecular testing in TS is long and laborious. Therefore, clinical diagnosis of TS is still a prerequisite to molecular testing.

P1060. Association between Type 1 Diabetes and myosin IX B (MYO9B) suggests that intestinal barrier integrity is important in disease susceptibility.

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We recently identified a strong association between the gene encoding for myosin IXB (*MYO9B*) and celiac disease (CD), and since type 1 diabetes (T1D) and CD share various clinical and epidemiological features, we have now investigated whether *MYO9B* is also associated with juvenile T1D. Three SNPs in the *MYO9B* gene were characterized for 288 T1D juvenile patients and 1615 control subjects. SNP rs2305767 was associated with T1D (p=0.03; OR 1.5, 95% CI 1.21-2.04). This association was confined to carriers of HLA DR-DQ genotypes (p=0.01), which predispose a moderate risk to T1D. Given the association of myosin IXB with CD, its implication in the integrity of the mucosal barrier, and the proposed role for dietary factors in the development of T1D, our results suggest the gut immune system plays a role in predisposition to T1D.

P1061. IL-18 and TNF-α promoter polymorphisms and susceptibility to type 1 diabetes in the Dalmatian population

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INTRODUCTION: Type 1 diabetes mellitus (T1DM) is characterized by a chronic progressive inflammatory autoimmune reaction that causes the selective elimination of pancreatic β cells. Proinflammatory cytokines, interleukin-18 (IL-18) and tumor necrosis factor alpha (TNF-α), both have been implicated in the pathogenesis of T1DM. Additionally IL-18 plays a role in regulation of TNF-α production. Therefore we tested an association of two single nucleotide polymorphisms (SNP) in promoter regions of these genes with the susceptibility to T1DM in the Dalmation population (South Croatia).
MATERIALS AND METHODS: 134 T1DM patients and 132 control subjects were tested for a G-137C change in a promoter region of IL-18 gene by a sequence specific PCR. TNF-α G-308A promoter variant was genotyped in a larger group of T1DM patients (206) and controls (144) by standard PCR followed with *NcoI* endonuclease restriction. Data were analysed using chi-square test.
RESULTS: A distribution of IL-18 G-137C and TNF-α G-308A promoter variants was equal between the T1DM patients and the controls (p=0.9448 and p=0.0655, respectively).
CONCLUSIONS: The present study provided no evidence of association of IL-18 and TNF-α promoter variants with susceptibility to T1DM in Dalmatian population. However as genotype frequencies for TNF-α G-308A polymorphism were close to the limit of statistical significance (p=0.0655) further analysis should be conducted.

P1062. Polymorphisms of some candidate genes in diabetic nephropathy in Romanian population

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Candidate gene studies have indicated that ACE (17q23), TGF-beta (19q13), VDR (12q13) and IGF2 (11p15) gene polymorphisms may be involve in DN onset or DN progression to ESRD.
The aim of the present work was to evaluate the impact of seven polymorphisms in these genes on renal failure in type 2 diabetic patients.
Clinical information and biological samples were collected from 99 (56M/43F) unrelated Romanian Caucasian subjects. The subjects were distributed into: H-T2DM (33 T2DM patients on hemodialysis, age: 58.5±7.8, diabetes duration: 18.1±10.7, duration of dialyses: 2.4±1.2), T2DM (33 nondialyzed T2DM patients, microalbuminuria < 30 mg/day, diabetes duration: 17.3±9.6) and C (33 healthy controls, fasting glycemia 93.2±8.2 mg/dl, microalbuminuria <30 mg/day) lots. These groups were matched for age, gender and ethnicity.
Each patient was genotyped for ACE ID, TGF-beta -800A/G, TGF-beta -509C/T, VDR Taq, VDR Apa, VDR Fok and IGF2 Apa polymorphisms by PCR or PCR-RFLP.
In contrast with some studies, we observed no difference between distribution of ACE ID, VDR Taq, VDR Apa, TGF- beta -800, genotypes and alleles in H-T2DM, T2DM and C groups (p>0.05). The TGF-beta -509CC (OR=2.4, CI: 0.8<OR<6.5), VDR FF (OR=2.0, 95%CI: 0.6<OR< 6.7) and IGF2 aa (OR=1.9, 95%CI: 0.7<OR< 5.5) genotypes were more frequent in H-T2D vs. C. From the present study, performed in relative small groups, we can conclude that the TGF-beta -509CC, VDR FF and IGF2 aa genotypes could predispose to the development of ESRD in Romanian T2DM patients

P1063. Transcript profiles of fat biopsies and two independent autopsy studies support the role of allelic variants of the *USF1*-gene in cardiovascular disease

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We recently reported association in Finnish families of the *USF1* gene with FCHL, a common dyslipidemia predisposing to cardiovascular disease (CVD). Expression and molecular studies were conducted in members of dyslipidemic families to investigate the functional relevance of different *USF1* alleles on target genes in adipose tissue. The role of specific *USF1* alleles was also addressed by quantitative analysis of arterial plaques in a human autopsy series.

The best associating SNP in the FCHL families is located in a conserved putative enhancer element in intron 7 that we established to bind nuclear proteins. In 19 fat biopsies, carriers of the risk allele of *USF1* presented with transcriptional changes in *USF1* target genes APOE, ABCA1 and AGT, all relevant to the pathogenesis of CVD.

To further establish the role of different *USF1* alleles on quantitative measures of arterial atherosclerosis in humans, we examined two autopsy series consisting of 700 males from the Helsinki area with sudden death, collected 1981-82 and 1991-92. The data included quantitative classification of atherosclerotic lesions in coronary arteries and abdominal aorta.

In the autopsy series, specific *USF1* alleles showed association with size of fibrotic intimal lesions, amount of calcification of the arteries and atherosclerosis of the brain (P=0.04 - <0.0001), as well as with death from ischemic heart disease (OR 5.00, 95% CI 1.75-14.30, P=0.003). These results underline the importance of variants of the *USF1* gene as a CVD risk factor at the population level and give more insight into the pathogenesis associated with these genetic variants.

P1064. Prevalence and distribution of exonic mutations in Interferon Regulatory Factor 6 (IRF6) identified in two large cohorts with Van der Woude syndrome

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Mutations in IRF6 cause Van der Woude (VWS) and Popliteal Pterygium syndromes (PPS), two related orofacial clefting disorders. IRF6 belongs to the IRF family of nine transcription factors. Both VWS and PPS display an autosomal dominant pattern of inheritance with high penetrance but variable expressivity. The phenotype of VWS includes pits in the lower lip, clefts of the lip and/or palate and hypodontia. In addition, PPS includes webbing of the lower limbs, syndactyly of the toes and digits, ankyloblepheron, oral synechia, and genital abnormalities. We performed direct sequence analysis on IRF6 exons on samples from two large geographically defined cohorts, one from Brazil (113 VWS and 1 PPS) and one of mixed origin (197 VWS and 36 PPS). We identified mutations in IRF6 exons in 69% of all families with VWS, and 97% of families with PPS. The distribution of VWS-causing mutations was not random, with exons 3, 4, 7, and 9 accounting for 80%. In total, we identified 87 protein truncation mutations, scattered throughout the gene, and 127 missense mutations that are concentrated in the DNA-binding and protein-binding domains. With 50% of the samples sequenced from the CEPH diversity panel, none of these mutations have been observed. In addition, PolyPhen and SIFT analyses of the missense mutations suggest that they are more likely to be damaging to gene function than all possible missense mutations (p<.001). This extensive mutation screen will assist clinicians to provide a DNA diagnosis for patients with orofacial clefts and to identify genotype-phenotype and structure-function relationships.

P1065. Vitamin D receptor FokI polymorphism is associated with knee strength in older women, not in men

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Introduction: A start codon polymorphism within the Vitamin D receptor gene (VDR, T to C) results in a three amino acids shorter VDR protein with increased vitamin D-related transcriptional activity. Gender-specific effects of this polymorphism have not been studied yet.

Purpose: To investigate the association between the *FokI* polymorphism in VDR and muscle strength in older men and women.

Methods: The *FokI* polymorphism (rs2228570, Sequenom MassARRAY SNP) was genotyped in 99 men (67.1+/-4.2yr) and in 102 women (66.4+/-4.5yr). The peak torque of knee extension and flexion was measured at velocities of 0°/s, 60°/s, 180°/s and 240°/s. Genotype-phenotype associations were tested using AN(C)OVA with muscle+bone cross-sectional area of the thigh as covariate.

Results: *FokI* genotypes were in Hardy-Weinberg equilibrium in both men and women (p=0.14 and p=0.07, respectively). In men no significant association was found between *FokI* and any of the measured strength phenotypes. In women, however, significant association was found with all measurements of knee strength except peak torque flexion at 0°/s and 60°/s. For most of the strength measurements the heterozygous group performed less well in dynamic knee strength than both homozygous groups (p<0.05).

Conclusion: The VDR *FokI* polymorphism is associated with knee peak torque in senior women but not in men, confirming the possibility of gender-specific effects. The lack of association in men confirms results of other research groups. As this is the first study to investigate the association between the VDR FokI polymorphism and knee strength in women, further research will be necessary to elucidate these findings.

P1066. Osteopenia molecular markers in children.

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The aim of our study was to investigate whether molecular markers, such as vitamin D receptor gene and osteocalcin polymorphism are related to osteopenia in children.

370 children (172 girls, 198 boys) with Insulin-depended diabetes mellitus, chronic arthritis and asthma were included in our study. The mean age of patients was 12,43±3,38 years. BsmI, ApaI, TagI vitamin D receptor gene (VDR) and osteocalcin gene HindIII polymorphism, serum levels of osteocalcin, β-CrossLabs, parathyroid hormone, Ca, phosphate, total alkaline phosphatase were tested in all patients. Osteopenia (OP) was detected by dual-energy X-ray absorptiometry in lumbar spine.

Using the data of BMD children were selected in two groups: with OP (Z score<-1,0 SD) - 67 (18,1%) and without OP (Z score>-1,0 SD) - 303 children (81,9%). We detected differences in TagI polymorphic genotypes (TT-18,2% and 39,9%, Tt - 45,4% and 50,0%, tt - 36,4% and 10,1%, p=0,002) and alleles distribution (T-40,9% and 64,7%, t - 59,1% and 35,3%, p=0,002) between all girls with and without osteopenia. Also, we detected differences in ApaI polymorphic genotypes (AA-57,1% and 23,4%, and Aa+aa-42,9% and 76,6%, p=0,05) and alleles distribution (A-78,6% and 47,7%, a - 21,4% and 52,3%, p=0,03) between girls 14-17 years with and without osteopenia. We revealed differences in HindIII polymorphic alleles distribution (H-40,0 % and 22,8%, h-60,0% and 77,2%, p=0,05) between children before 10 years, and between girls 11-13 years (H-33,3% and 13,8%, h-66,7% and 86,2%) with and without osteopenia, consequently. We conclude that these polymorphic molecular markers are suitable for detecting osteopenia genetic predisposing.

P1067. An investigation of eight candidate gene polymorphisms in South Asian and Caucasian RA patients of the East Midlands in UK.

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Rheumatoid Arthritis (RA) is a chronic musculoskeletal disease of unknown aetiology. RA is a complex polygenic and multifactorial disease and has not been analysed comprehensively among South Asians, specifically in the East Midlands.

Two genetic approaches were used; case-control and sib-TDT analyses. Ten polymorphisms of eight genes (ACE, VDR, A2M, GSTT1 and GSTM1, TNFRII, FcgRIIIA and CRH) were analysed in South Asians (134 patients, 66 unaffected sibs, 149 random controls) and Caucasians (137 patients, 83 unaffected sibs, 150 random controls). The gender distribution (male:female) was 1:4 in South Asians and 1:3 in Caucasians.

Significant genetic associations were observed with VDR *Bsm I* B-B genotype (OR = 2.08, CI 1.23 - 3.52, P < 0.05), A2M 2-2 genotype (OR = 3.99, CI 1.19 - 17.18, P < 0.05), and GST T_1null genotype (OR = 2.81, CI 1.40 - 5.77, P < 0.002) among South Asian RAs. In Caucasians, TNFRII R-R (OR = 3.16, CI 1.20-9.26, P < 0.05), A2M 1-1 (OR = 2.09, CI 1.21-3.64, P < 0.05) and GST T_1null (OR = 1.97, CI 1.07 - 3.68, P < 0.05) genotypes were associated with RA. In the majority of cases, recessive and multiplicative modes of inheritance explained the observed associations. There were no confounding interactions between the genotypes.

Overall this study demonstrates that ethnic and genetic variation plays a significant role in RA susceptibility.

P1068. Mutations R67X and W303X of the protein Z-dependent protease inhibitor gene are not associated with venous thromboembolic disease.

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The protein Z-dependent protease inhibitor (ZPI) is a serpin that inhibits the activated coagulation factors X and XI. Previous investigations indicated that no association is present between plasma levels of protein Z and ZPI and venous thrombosis. However, it has been recently shown that two nonsense mutations (R67X and W303X) of the *ZPI* gene are associated with venous thromboembolic disease (VTE) in New Zealand (1).

Aim of this study was to investigate the presence of the R67X and W303X mutations of the *ZPI* gene in VTE patients and control subjects in a population of North-East Italian region.

DNA samples were collected from 183 consecutive VTE patients and 113 normal controls. The R67X and W303X mutations were detected by bidirectional allele-specific PCR, according to the method published by Van de Water et al. (1).

The R67X mutation was not present in any of the VTE patients or control subjects. The W303X mutation was present in two VTE patients (1,1%) and one control (0,85%). This difference was not statistically significant. Thus, our data support the notion that the R67X and W303X mutations of the *ZPI* gene are rare gene variants in European populations and are not associated to VTE, Our findings confirm results of a previous study made in Spanish subjects (2).

1. van de Water B., et al. British J. Haematol, 127: 190-194, 2004.
2. Gonzales-Conejero R., et al. British J. Haematol, 129 : 561, 2005.

P1069. Linkage study in four large Dutch families with VUR: exclusion of a previously reported locus and (separate) candidate genes

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Vesico-ureteral reflux (VUR [MIM 193000]), the retrograde passage of urine from the bladder, is one of the most commonly detected congenital anomalies. It has a prevalence of 1%. VUR is a major cause of urinary tract infections in children and is the cause of 7% of end-stage renal disease in pediatric patients in the Netherlands. Genetic factors play an important role in the etiology of primary VUR since (1) sibs of affected children have a 32% risk of VUR, and (2) there is 80% concordance between monozygotic twins.

A locus on 1p13, previously shown to be linked to vesico-ureteral reflux (VUR), still awaits replication. To investigate the involvement of this locus and 9 (separate) candidate genes for VUR in four multi-generation Dutch VUR families, we performed a linkage study using 56 individuals, including 21 patients. While verifying the physical location of the two flanking markers of the 1p13 locus, we noticed that they now have different map locations on chromosomes 1q23 and 2q11. We were unable to detect linkage with a total of 18 microsatellite markers markers covering both loci. Hence, we were able to exclude the (1p13 to) 1q23 locus and 61% of the 2q11 locus from linkage to VUR. We were unable to detect linkage to any of the candidate genes either. Our results imply that neither the adjusted 1p13 locus, nor any of the candidate genes we tested, plays an important role in Dutch families with VUR.

P1070. The influence of vitamin D receptor polymorphisms on bone mineral density and bone turn over

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Aims: To determine association of vitamin D receptor (VDR) gene polymorphisms with bone mineral density (BMD) and bone turn over.

Methods:We studied allelic frequencies of the FokI,BsmI, ApaI, and TaqI restriction fragment length polymorphisms in 38 familial osteoporotic patients in comparison with 40 age-matched healthy pre and post menopausal women , and correlated their bone mass with the VDR genotypes. Genomic DNA was isolated from peripheral blood leukocytes according to standard methods. After an overnight fast, blood was taken for measurement of serum parathyroid hormone, 25-hydroxyvitamin D, alkaline phosphatase, alkaline phosphatase, osteocalcin and cross laps. Bone measurements were performed with the same instrument in two regions. Calcium and vitamin D intake estimated from a detailed food recall interview for the previous month.

Results:The most common VDR genotypes were Aa (52.5 percent), Bb (37.1 percent), FF(52.5 percent), and Tt (47.4percent). There were statistical differences in the allelic distribution of FokI and TaqI between osteoporotic patients and controls. After adjustment for age, BMI, calcium and vitamin D intakes, statistical associations were found between TaqI VDR gene polymorphisms and BMD and were weakly correlated with serum concentrations of osteocalcin, alkaline phosphatase. The vitamin D-receptor gene alleles in other three genotypic groups were not associated with the serum concentrations of calcium or other biochemical values, calciotropic hormones, or markers of bone turnover.

Conclusions: VDR gene alleles predict the bone density and bone turn over .Our results showed that a significantly differences in frequency of the VDR allelic distribution in comparisons with the Asian population.

P1071. A novel locus for primary familial vesicoureteral reflux maps to chromosome 3q

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Vesicoureteral reflux (VUR) is the most common urological disorder in children and refers to the retrograde flow of urine from the bladder into the kidneys. Commonly, VUR occurs as a primary entity [OMIM 193000] and affects 1% to 2% of the Caucasian population.

In order to identify gene(s) involved in the pathogenesis of primary VUR, 150 patients were recruited and clinically characterized. Diagnosis and grading of VUR were done according to the International Grading System using standard techniques. Available family members were ascertained and investigated for the presence of reflux, reflux nephropathy or renal failure. Blood samples were collected and DNA was isolated following standard procedures.

A genome wide search was performed in 14 Italian families with 49 index cases with primary VUR, showing a pattern of inheritance compatible with an autosomal dominant model. The statistical analysis (Genehunter, non-parametric analysis) highlighted 4 genomic regions on different chromosomes (NPL>2.5, p<0.015) that might contain gene(s) implicated in the etiology of VUR. None of the families was linked to the chromosome 1p locus reported previously. Interestingly, the majority of the families (n=10) support a single novel region on chromosome 3q (max NPL=2.69, p=0.006). Therefore, the region of interest was tested in 11 additional families with a total of 78 patients. Non-parametric analysis confirmed the presence of a novel VUR locus on chromosome 3q (NPL=2.84, p=0.004). The best part of the region supported by the linked families spans 3 Mb and contains 15 genes, currently subjected to sequence analysis.

P1072. A 300K Phase I HapMap Tag SNP Panel

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We have developed a panel of >317,000 SNP loci chosen from the Phase I HapMap data (www.hapmap.org). We use a novel whole genome genotyping assay to interrogate this large number of SNPs efficiently and accurately on a single slide. The assay uses a PCR-less single tube whole genome amplification step, array hybridization capture, and an array-based primer extension reaction to directly score the captured SNP targets (Gunderson *et al*., 2005; Steemers *et al*., 2006).

A maximally informative set of tag SNPs were derived from the Caucasian (CEPH) population and their utility has been assessed in the Han Chinese, Japanese, and Yoruba populations. Tag SNPs were chosen using algorithms utilizing the linkage disequilibrium statistic r^2 (Carlson *et al*., 2004). A higher density of tag SNPs within 10 kb of genes or in evolutionarily conserved regions were chosen using a more stringent r^2 threshold. In addition, we have included ~8,000 nsSNPs and >1,000 tag SNPs chosen from a 2 kb map of SNPs across the MHC region. This panel captures 80%, 68%, and 34% of HapMap Phase I+II variation in CEPH, Han Chinese/Japanese, and Yoruba populations at $r^2 \geq 0.8$, respectively. The average spacing between SNP loci is 9kb (median 5 kb; 90th percentile 19kb). This panel of tag SNPs will provide a valuable resource for whole genome genotyping studies to identify the genetic variation involved in health and disease.

P1073. Identification of genetic variants in the ATP7B gene promoter and 5'UTR in Wilson disease patients

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Wilson disease is an autosomal recessive disorder that affects intracellular copper transport, resulting in copper accumulation in some tissues and leading to hepatic and/or neurological damage. Molecular analyses of the ATP7B gene in Wilson disease patients have identified more than 200 different alterations, some of them affecting the regulating regions of this gene. We used SSCP analysis to investigate the 5'UTR and promoter of the ATP7B gene in 40 WD patients, in whom our previous molecular analysis of the coding region and intron/exon boundaries had failed to identify one or both mutations. Seventeen of the 40 samples presented an altered SSCP pattern. Sequencing analysis of these samples is now on course. Until this moment, one patient has been found to be homozygous for the -525T>C substitution and heterozygous for the -413T>C one. These two single base changes have been identified previously in other studies and considered as probable variants with no clinical effect after detecting them in the normal population. In another patient, we have identified a novel C to G base change in heterozygous state in the -401 position. Screening studies of the control population are in progress in order to discriminate between a normal variant or a possible disease-causing mutation. The common -75A>C substitution and the -132delGCCGC deletion have been identified in several of our patients, but we did not consider them as disease-causing mutations since they have been found in the normal population in diverse studies.

P1074. Mutational Screening of the RP2 and the RPGR Genes in Spanish Families with X-Linked Retinitis Pigmentosa

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Purpose: X-linked form of Retinitis pigmentosa (XLRP) is one of the most severe types of RP. Five XLRP loci have been mapped although only two genes, *RPGR* and *RP2*, have been cloned.

We screened 30 unrelated XLRP Spanish families in order to determine the molecular cause of their disease.

Methods: We have performed haplotype analysis and, in those families in which the disease segregates with the RPGR and/or the RP2 locus, we carried out mutational screening. We have analyzed the *RP2* gene, the first 15 exons of *RPGR* at cDNA level and the ORF 14 and ORF 15 exons at genomic DNA level.

Results: After haplotype analysis we could rule out the implication in the disease of *RP2* and RPGR in 6 and 4 families, respectively.

Among the 30 unrelated XLRP, we found 16 mutations in *RPGR* (7 are novel) and 4 mutations in *RP2* (3 are novel).

Conclusions: In our cohort of XLRP families RPGR also seems to be the most prevalent form of XLRP.

Based in our results we propose a protocol for molecular diagnosis of XLRP families, in four consecutive steps:

1) Haplotype analysis

2) In case that haplotype was not informative, we propose to analyze exon ORF-15 of RPGR gene.

3) Later the first 15 exons of gene RPGR.

4) And finally the 5 exons of gene *RP2*, in the last two cases using mRNA.

We consider that this approach is the most effective method (rapid and accurate) for mutation screening in XLRP cases.

P1075. XRCC3 Thr241Met polymorphism and CNS cancer in a Spanish population

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Hereditary genetic defects in DNA repair lead to increased risk of cancer. Genetic polymorphisms that influence individual response to environmental exposures can contribute to cancer susceptibility.

To find a possible association to brain cancer susceptibility, we analyzed both genotype and allele frequencies of XRCC3 Thr241Met polymorphism from DSB pathway in brain cancer patients.

We have studied 230 individuals, previously diagnosed with brain cancer and 393 healthy controls.

Analysis of allele distribution in XRCC3 Thr241Met showed a greater representation of the Thr allele in the group of patients when compared to the controls (p=0.000) and it is associated with an increased cancer risk (OR= 2.653, 95% CI=2.007-3.507).

POLYMORPHISM	n		GENOTYPE		P value
XRCC3 241		***Thr/Thr***	***Thr/Met***	***Met/Met***	
Controls	385	70 18.2%	174 45.2%	141 36.6%	
Patients	147	63 42.9%	64 43.5%	20 13.6%	**<0.001**[1]
Meningiomas[a]	55	24 43.6%	25 45.5%	6 10.9%	**<0.001**[2]
Gliomas	92	39 42.40%	39 42.4%	14 15.2%	**<0.001**[3]

[a] Difference of genotype frequencies. Meningiomas vs. gliomas p=0.757
[1] Controls vs. Patients
[2] Controls vs. Meningiomas
[3] Controls vs. Gliomas

Table 1. Genotypic frequencies of the XRCC3 241 polymorphism in brain cancer patients and controls

Our results suggest that the presence of the Thr allele increases the susceptibility to develop brain tumors whereas the Met allele may be considered as a protective factor.

P1076. Specific RET polymorphisms contributing to Hirschsprung patient's phenotype

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Hirschsprung disease (Hd) is a congenital heterogeneous disorder, characterized by the absence of intestinal ganglion cells. In aetiology of Hirschsprung disease various genes are included. It seems that the most important role in its aetiology plays *RET* gene. There are plenty of different mutations in this gene. No mutation is fully penetrant and they have varying effects on the length of the aganglionic segment of the intestine. The aim of our study was to analyse single nucleotide polimorphisms (SNP) of *RET* gene in exon 2, 3, 7, 11, 13, 14 and 15. To test how the Hd phenotype may be affected by the presence of genetic variants, we compared the molecular results with clinical and long-term follow-up data. Molecular DNA analyses were performed in 70 patients with Hd. We found a short and ultra-short segment of aganglionic gut in 84,3%, and long-segment in 15,7% of all patients. The 135G/A *RET* polymorphism in exon 2 was over−represented in Hd populations compared to normal, unrelated control individuals. Two other polymorphisms: 2071G/A (exon 11) and 2712C/G (exon 15) were under−represented in the population of Hd. Moreover, the 135G/A *RET* variant has been strongly shown to be associated with the Hd phenotype. We have demonstrated that *RET* haplotypes containing these polimorphisms play a role in the aetiology of Hd. While the allelic variant in exon 2 can predispose to Hirschsprung disease development, it seems that tested polymorphisms in exon 11 and 15 could have a protective effect against the severe type of aganglionosis.

P1077. Secret of the short life span: smoking and complement interact in promoting cardiovascular disease morbidity and mortality

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Smoking carries a major risk for cardiovascular disease (CVD). We have determined the frequency of the so-called silent allele of the complement C4B gene (C4B*Q0) (less C4B than C4A genes in the genome) in 263 and 233 patients, respectively, with different types of coronary artery disease and in 479 and 274, healthy subjects, respectively from Iceland and Hungary. .Smoking habits were registered at all subjects tested. We found that C4B*Q0 confers an increased risk of angina pectoris (AP), myocardial infarction (MI) and MI-associated mortalities in smokers. C4B null alleles were raised at diagnosis in smokers with AP (p=0.02) and MI (p=0.001), but conspicuously absent in patients who survived a MI and continued to smoke for a period exceeding 3 years. The risk of MI short-term (<6 months) mortality was much higher in C4B*Q0 carriers than in non-carriers (adjusted odds ratio: 17.77, p=0.003). In non-smokers no increase was observed in C4B*Q0 at MI or AP. The age-associated decrease in C4B*Q0 previously observed in two remote Caucasian populations was in the present study found to be strongly associated with smoking and to occur already at age 50. C4B*Q0 can now be identified as a major covariate of smoking in precipitating the risk for MI and associated deaths. These results may help to identify new possibilities for future research on the pathophysiology of CVD as well as for development of novel prophylactic or therapeutic intervention strategies

Po07. Normal variation, population genetics, genetic epidemiology

P1078. Mitochodrial DNA variability in the Kazakh population of Middle Asia

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Information about mtDNA variation in Middle Asia is very crucial for understanding of Asian mtDNA phytogeny as well as for reconstruction of Asian population history. The sequence of the first hypervariable segment of mtDNA was determined in 246 individuals from three district of Kazakhstan. 120 polymorphic positions and 192 different HVS 1 haplotypes were revealed. For correct haplogroup affiliation the ambiguous HVS 1 sequences were additionally screened for RFLP markers. 38 gaplogroups of mtDNA was determined. HVS 1 haplotypes occuring once (unicum haplotypes) were determined 64,6% in Kazakhs. Unicum haplotypes is different values in the populations of the Volga-Ural region: for Chuvaschs --64%, for Bashkirs-88%, for Tatars -80%.

Index variability of gaplotypes is 0,99 in Kazakhs. Index variability of gaplotypes are analogical values in the populations of the Volga-Ural region: for Chuvaschs and for Bashkirs-0,98, for Tatars -0,99.

Index of gene diversity (calculated about of the frequencies gaplogroups) is 0,93 in Kazakhs. From literature it is known that the same values index of gene diversity in population for Kirgizs - 0,87, for Uzbeks - 0,92, Tajiks - 0,85 [Golubenko et.al., 2002].

The populations of kazakhs have generally higher values of gene diversity of mtDNA by complicated ethnic history of the Kazakh population. The comparison of Kazakhs and other ethnic origin reveals the complicated structure of mtDNA gene pool in Middle Asia.

P1079. δ-thalassaemia in Cyprus

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To help clarify the haematological picture of patients who may be positive for β- and δ-globin gene mutations, the following study was carried out aiming to identify the δ-globin gene mutations found in the Greek Cypriot population, their frequencies and the HbA_2 values associated with them. 74 samples were selected from a random sample of 5030 individuals and the database of the Molecular Genetics Thalassaemia Department containing diagnostic analyses data was also mined for relevant information.

Three novel for Cyprus δ-globin gene mutations (-30, IVS-I-2, HbA_2-Yohoshima) were identified, bringing the total of δ-globin alleles in the Greek Cypriot population to ten: HbA_2-Yialousa, HbA_2-Yokoshima, HbA_2-Troodos, HbA_2-Pelendri, Codon 4, Codon 59, IVS-II-897, IVS-I-2, -55, -30. HbA_2-Yialousa is the most common mutation with a frequency of 61.1% followed by Codon 4 (frequency 20.4%).

HbA_2 levels over 1.9% have been found to indicate a significantly reduced possibility for the presence of a δ-globin gene mutation in this population (≤20%). For HbA_2 levels below 1.9%, the possibility of a

heterozygote δ-globin gene mutation rises to 80-100%.
The frequency of all the mutant δ-globin alleles in the sample is 0.0067 and the carrier frequency is 1.26%.

P1080. ABO-genotyping by means of gel-based DNA microchips

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A method of ABO alleles discrimination using microchip technology is presented. Gel elements of the microchip contain SNP-specific oligonucleotide probes able to reveal polymorphisms in positions 261, 297, 526, 646, 657 and 681 of ABO-locus. The amplification of exon 6 and 7 was carried out in multiplex nested PCR with fluorescently labeled primers. Then hybridization on a chip was performed and the ABO alleles were detected according to haplotype-specific fluorescence of gel elements. 75 DNA samples of residents mainly from the Central region of Russia were investigated. The following blood groops distribution was obtained: I-38.4%, II-29%, III-28%, IV-4.6%. The results of allele determination were the following: A-20.3%, B-19.2%, O^1-37.8%, O^{1v}-21.5%, O^2-1.2%. To confirm the accuracy of genotyping 10 samples were sequenced and the results were verified.
The sensitivity of the method amounts to 15 pg of DNA for a single test which is equal to genome of 3 somatic cells. Due to high sensitivity the method will be promising in identification of individuals especially in complicated cases. Furthermore, the method is able to distinguish 15 genotypic blood groops instead of 4 serologic ones that would increase a discrimination power of forensic examination.

P1081. Genetic polymorphisms as basic predictive markers of aging

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The polymorphisms of 15 genes (AGT, ACE, AGTR1, PLAT, PAI1, GPIIIa, MTHFR, NOS3, MTRR, GSTT1, GSTM1, VDR, COL1A1, CALCR, ER1) responsible for multifactorial diseases were studied in three different groups. The newborn group included 102 unrelated individuals of newborn age, middle-age group included 122 unrelated individuals of middle age (25-45), the group of elders - 148 unrelated individuals over 70 years. Progressive increasing of frequencies of M/T (AGT) genotype in the elderly group as compared to the middle age (56.0% and 34.0%, respectively, p=0.0006) and I/D (ACE) genotype in the row newborn-middle-age-elderly (41.3%, 45.4%, 50.3%, respectively) was registered. The frequency of A/C (AGTR1) genotype decreased in the elderly men compared with middle age (31% and 51%). The frequency of GSTT10/0 genotype was different in studied groups (newborn group - 18.4%, middle-age - 22% and elderly people - 28%). The frequencies of deletion homozygotes (GSTT10/0 and GSTM10/0) gradually increased with aging 6.7%, 14.5% and 16.0% in newborns, middle-age and elderly people (after 90 years) respectively. The frequencies of genotypes and alleles of other genes were similar in the studied groups. However, combined analysis revealed significant prevalence of genotype I/D (ACE), 4a/4b (eNOS), C/C (MTHFR) in elderly people compared to middle-age ones (p=0.035). So, it might be speculated, that some alleles of these genes are associated with life span. Further, it is necessary to perform studies on various groups of different age, and take into account meta-analysis of the data from other laboratories to estimate the role of some genes in aging.

P1082. *ADH2* polymorphism in mountain and steppe populations

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ADH2 is one of the major enzymes metabolizing alcohol. This enzyme is encoded by ADH2 gene, which contains two functional polymorphisms. Arg47His enzyme (ADH2*2) is superactive, leading to more rapid accumulation of acetaldehyde. It has been documented that there are differences in frequencies of ADH2 allels between populations.
The analysis of ADH2 gene was performed by PCR method. The population samples included: 114 Russians (belongs to Slavic group of Indo-European language family); 57 Tatars, 66 Bashkirs, 40 Balkars (Turkic group of the Altaic language family); 53 Circassians, 50 Abkhazes (Western group of the North Caucasus language family); 50 Avars (Eastern group of the North Caucasus language family).

Populations	N	Genotype frequency			Allele frequency	
		1/1	1/2	2/2	1	2
Russians	114	0.96	0.04	0	0.98	0.02
Tatars	57	0.93	0.07	0	0.96	0.04
Bashkirs	66	0.88	0.008	0.04	0.92	0.08
Circassians	53	0.75	0.23	0.02	0.87	0.13
Balkars	40	0.75	0.23	0.02	0.86	0.14
Abkhazes	50	0.82	0.16	0.02	0.90	0.10
Avars	50	0.66	0.30	0.04	0.81	0.19

By geographical localization, the Russians, the Bashkirs and the Tatars belong to steppe populations, while the Circassians, the Balkars, the Abkhazes and the Avars belong to mountain populations.
We found the frequency of ADH2*2 was significantly different among all examined populations (chi2=35.25, p<0.001) and was ranged from 2% in Russians to 19% in Avars. In total mountain populations were characterized by higher frequency of ADH2*2 allele, compared to that of steppe populations (chi2=25.82, p<0.001).
According to our data ADH2*2 allele has higher frequency in mountain populations. We can suggest that because of hard climatic conditions, high alcohol consumption and high mortality natural selection led to surviving of individuals with lower risk for alcoholism.

P1083. Analysis of common alpha-thalassemia point mutations and deletions by reverse-hybridization

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Alpha-thalassemia (alpha-thal) is observed in high frequencies throughout Southeast Asia, India, the Middle East, parts of Africa and the Mediterranean area. It is characterized by the reduced synthesis or absence of alpha-globin chains due to mutations affecting one or both genes. The clinical phenotype varies from asymptomatic to lethal (Hb Bart's hydrops fetalis) according to the number of impaired alpha-globin genes.
We have developed a reverse-hybridization assay (Alpha-Globin StripAssay) for the rapid and simultaneous detection of 21 alpha-globin mutations: two single gene deletions (-3.7; -4.2), five double gene deletions (MED; SEA; THAI; FIL; -20.5 kb), anti-3.7 gene triplication, two point mutations in the alpha 1 gene (cd 14: TGG-TAG; cd 59: Hb Adana GGC>GAC) and eleven point mutations in the alpha 2 gene (init cd: ATG>ACG; cd 19: -G; IVS1: 5nt del; cd 59: GGC>GAC; cd 125: Hb Quong Sze CTG>CCG; cd 142: Hb Constant Spring TAA>CAA; cd 142: Hb Icaria TAA>AAA, cd 142: Hb Pakse TAA>TAT; cd 142: Hb Koya Dora TAA>TCA; poly A-1: AATAAA-AATAAG; poly A-2: AATAAA-AATGAA). The test is based on multiplex DNA amplification (including gap-PCR) and hybridization to teststrips presenting a parallel array of allele-specific oligonucleotide probes for each variant. The entire procedure from blood sampling to the identification of mutations requires less than 6 hours, and hybridization/detection may be carried out manually or essentially automated using existing instrumentation (e.g. TECAN profiBlot). The Alpha-Globin StripAssay has been carefully validated, both on pre-typed reference samples, as well as in routine diagnostic settings. (oberkanins@viennalab.co.at)

P1084. Alzheimer's Disease and the Cystatin C gene polymorphism: an association study.

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Cystatin C is a cysteine protease inhibitor which is found to colocalize with Aβ in plaques and cerebrovascular deposits in Alzheimer's Disease (AD). Recent studies have reported a genetic association between the 73 G/A polymorphism within exon 1 of the cystatin C gene (CST3), a common Ala/Thr substitution in the signal peptide, and Alzheimer's disease (AD) with conflicting results. To further investigate the proposed association in our population, we analyzed this variant in a clinic and population based group of 171 Italian patients with sporadic AD from southern Italy (Calabria region) and 190 healthy controls subjects from the same geographical area. All 361 subjects were genotyped for CST3 and APOE polymorphisms but our data showed no association between AD and CST3. We therefore stratified our samples based on age (of controls) or age of onset (of cases): <65-69, 70-79, and 80+ years. After this stratification according to age, in older patients (80+ years) the GG frequency resulted over-represented when compared to controls, but far from statistically significant. There was also no evidence of a statistical interaction between CST3 and APOE polymorphisms. In conclusion, our data suggest that the 73 G/A polymorphism within exon 1 of the cystatin C gene is not a susceptibility factor in AD and nor mitigate the effect of the ApoE -ε 4 allele in the risk of developing AD in our population but further studies will be necessary to clarify the CST3 polymorphism position among AD risk factors.

P1085. Two SNPs in the Fas gene on chromosome 10 are not associated with Italian Sporadic Alzheimer's Disease

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The TNFRSF6 (Tumor Necrosis Factor Receptor Super Family 6) gene encodes Fas antigen, a cell surface receptor-mediating cell apoptosis situated on chromosome 10q, near the region of linkage to sporadic Alzheimer's Disease (AD). Moreover, elevated levels of Fas have reported in the brain of AD patients. These two criteria, positional and pathobiological, make the Fas antigen an interesting candidate for an association with AD. To address these findings, we have tested two SNPs in the TNFRSF6 gene in a set of 223 Italian patients with non-familial (sporadic) AD from southern Italy and 211 healthy controls subjects: a G to A polymorphism at position (-670) in the enhancer region of the promoter and a single nucleotide change from C to T 74 nucleotides from the beginning of exon 7 in the Fas gene. There was no statistically significant differences in allelic and genotypic frequency distribution between cases and controls or between late and early-onset AD patients. No interactive effect was found between the Fas polymorphisms and the known risk factor of non-familial AD, Apolipoprotein-E ε4 allele. We also tested whether these different Fas genotypes were associated with clinical features, such as age at disease onset and disease progression but no significant differences was detected. The present data suggest that the polymorphisms do not represent a risk factor for AD in our population.

P1086. Effect of Apolipoprotein E (APOE) genotype and past fertility on age of onset of Alzheimer's disease in women

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Various genetic and non-genetic risk factors have been linked to the development of Alzheimer's disease (AD). In women, having had children is one of the non-genetic factors reportedly associated with an increased risk of AD. Among genetic factors involved in AD susceptibility, the APOE e*4 allele has a major role and its presence reduces age at AD onset. But APOE is also thought to influence human reproduction as well, and common APOE genotypes seem to be associated with differential fertility. With this study, we investigated possible relationships between APOE genotype, past fertility, and AD onset age in a sample of 116 women with the sporadic form of the disease. Results from a comparison of APOE genotype distribution in parous and nulliparous AD women supported previous findings indicating that the e*3/e*3 genotype was associated with higher fertility and the e*4 carrying genotypes with lower fertility. When the combined effects of fertility and APOE genotypes on AD onset age were analysed parity was found to be associated with a significantly lower AD onset age (72.3±5.9 years) than nulliparity (79.0±5.6 years; P=0.004) among e*3/e*3 homozygotes. Since e*3/e*3 is the most frequent APOE genotype in Europe (0. 56--0.70), past fertility may influence AD susceptibility in many women. A similar effect was absent among e*4 carriers. Our findings indicate that past fertility may have a relevant effect on AD onset age and that the effect is mediated by APOE genotype.

P1087. Complex haplotypes from apo(a) gene locus control regions and correlation to Lp(a) plasma levels

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Objectives: High lipoprotein(a), Lp(a), level is an independent risk factor for developement of premature atherosclerosis. Apolipoprotein(a), apo(a), is the main determinant of Lp(a) plasma concentration. The aim of our study was to reconstruct haplotypes using five polymorphisms from the apo(a) gene regulatory sequences (promoter, DHIII enhancer) and to compare their distribution in five groups of individuals with different range of Lp(a) level and in a population sample.
Methods: The out-patients pool of the 3rd Medical Department was divided into quintiles according to Lp(a) concentration. Population sample was derived from the Institute of Biology and Medical Genetics. Three polymorphisms (TTTTAn repetition, +93C/T, +121G/A) from the apo(a) gene promoter and two polymorphisms (-1617C/A, -1230A/G) from the DHIII enhancer region were detected by the fragmentation analysis (TTTTAn repetition) and by the DGGE method in combination with sequencing in the quintiles and by the RFLP and allele-specifice PCR in the population sample.
Results: From the 80 possible haplotypes 23 were able to bulit up all observed genotypes in the quintiles and population sample. Several statistically significant differences were observed in frequencies distribution among quintiles and between each quintile and population sample. Some of the haplotypes were isolated to a narrow range of Lp(a) levels.
Conclusion: We conclude from our study that complex haplotypes from apo(a) gene locus could be used as a marker for certain range of apo(a) gene length variants and thus Lp(a) levels.
This study was supported by grant IGAMZCR NR-8328-3/200 and by MSM0021620807.

P1088. Polymorphism of the ApoE locus in the Azores Islands (Portugal)

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The aim of this work was to report on the polymorphism of the ApoE locus in the Azores Islands (Portugal) in order to get insights on the genetic background that influences the lipid profile in this population, ascertained as considerably high in a preliminary study of a sample of healthy subjects (60% of individuals were hypercholesterolemics). One hundred and twenty six Azorean individuals were typed for ApoE polymorphism using standard PCR-RFLP. The allelic frequencies obtained for ε2, ε3 and ε4 were 6.75%, 83.73% and 9.52%, respectively. Genotypic frequencies were in conformity with Hardy-Weinberg expectations. The ε3/ε3 genotype presented the highest frequency (69.84%), whilst ε4/ε4 was the least frequent (0.79%). The genotypic and allelic frequencies observed were similar to those reported for other Iberian samples. Furthermore, Nei's gene diversity (0.2864±0.0351) was similar to the reported for samples from Mainland Portugal. Results obtained did not evidence a particular behaviour of the ApoE locus that could be directly related with the high levels of total cholesterol determined in the Azorean population.

P1089. Birth defects registry in a region of Plovdiv, Bulgaria, 18-years experience

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Registration programs of congenital anomalies carry out a systematic collection of congenital anomalies among newborn children and stillborn and perform analyses of these data. Here we present a study, which includes the data of congenital anomalies registry program for the region of Plovdiv, Bulgaria, during the period of 18 years, 1987-2004. The study comprises 84162 newborn with 1753 registered congenital anomalies and inherited disorders among them (2,08%) The number and the proportion of various groups of anomalies towards all newborn as well as all children with anomalies are presented. The greatest part of all anomalies represent the syndromes with multiple anomalies, 16,54%, followed by congenital heart defects, 15,86%; limb anomalies, 11,69%; open neural tube defects, 9,64%; chromosomal disorders, 8,96%. Monogenic disorders and syndromes are resumed separately. The parallel analysis between the results of this study and those of other European registries disclose a not fully sufficient application of prenatal screening methods among pregnant women especially ultrasound diagnostics of fetal morphology in the region of Plovdiv.

P1090. The distribution of the C18T and G36T genetic variants of the ADP platelet receptor gene P2Y12 in octogenarians and newborns from Russia

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ADP is the most important mediators of haemostasis and thrombosis. Effect of ADP on platelets is mediated by two receptors - P2Y1 and P2Y12. The P2Y12 receptor plays a key role in platelet aggregation. Recently two new SNPs in coding region of P2Y12 gene were found in Russian population - C18T and G36T. We had analyzed these genetic polymorphisms in the case-control study for myocardial infarction and conclude that C18T may prevent platelets hyperaggregability and play a protective role in myocardial infarction development in contrast G36T may be a risk factor for myocardial infarction. For confirmation this hypothesis the frequencies of C18T and G36T genotypes were investigated in two groups - 132 healthy volunteers older 85 years without any thromboembolic events or myocardial infarction and 155 newborn infants. For detection of the C18T and G36T variants the polymerase chain reaction and original endonuclease digestion with MnlI and SecI were used. We found the prevalence of the 18T allele (carriers of 18CT and 18TT genotypes) in volunteers older 85 years compared newborn infants - 65% and 54%, respectively (p=0.04), and the relative decrease of the 36T allele (carriers of 36GT and 36TT genotypes) in healthy volunteers - 23% compared with newborn infants - 32% (p=0.1). The group of newborn infants reflects the distribution of genetic variants in our population but in the healthy volunteers older 85 years we may reveal the protective alleles. We conclude that C18T P2Y12 play a protective role in development of thromboembolism or myocardial infarction.

P1091. Analysis of C282Y and H63D mutations of HFE gene in populations of Central Asia

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Hemochromatosis is a hereditary disease of iron metabolism disturbance. Identification of the hemochromatosis-causing genetic defect will provide early diagnostics, population screening for the carrier state, as well as the performance of medical genetic counseling. With the aim of identification of heterozygous carriers and establishment of the disease screening pattern, as well as for the description of the gene pool ethnic features, the C282Y and H63D mutations in the HFE gene were examined 594 individuals from 5 populations of Central Asia belonging to Turkic group of Altaic language familty: Kazakhs, Uzbeks, Kirgizis, Turkmens, and Uighurs, and from 2 populations belonging to Iranic group of Indoeuropean language family: Tadjiks, and Kurds. C282Y mutation has been found in Uighurs (0.009), Kazakhs and Tadjiks (0.012), but hasn't been found in other populations. H63D mutation has been revealed in all investigated populations with frequency ranging from 0.024 in Tadjiks to 0.139 in Turkmens. The obtained results demonstrate that Central Asian populations are genetically situated between European and Eastern-Asian populations, corresponding with Central Asia geographic location. There was no population differentiation both in general and relative to the population linguistic attribution, neither at the individual loci examined, nor over the two loci. The revealed low frequency of the C282Y mutation (< 1%), which is the most functionally important of all known HFE mutations for the development of hereditary hemochromatosis type 1, reduced the attractivity of molecular genetic screening of the disease in the risk groups in this region as an effective diagnostic tool.

P1092. An age-associated decrease in the frequency of C4B*Q0 and C4A*Q0 genotypes in Hungarian general population

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The C4 complement protein is encoded by two genes (C4A and C4B) in the MHCIII region. In about 50% of Caucasian population the number of the C4A and C4B genes is equal while in about 25%-25% the number of C4A genes is lower (C4A*Q0) or higher (C4B*Q0) than that of the C4B genes. Previously we found the C4B*Q0 genotype associated with short life expectancy, while C4A*Q0 genotype, member of the 8.1 ancestral haplotype (8.1 AH) was strongly linked to ever smoking trait in women. We wanted to confirm these observations by a new RT-PCR based method. The sample group representing age and sex distribution of the Hungarian general population was randomly recruited. We determined Hsp70-2, TNF -308 SNPs, and the number of C4A/C4B genes.

Samples were from three age groups (30-39 years, 60-69 years, 70-79 years). In the two younger age groups the percentage of the C4A=C4B, C4A*Q0 and C4B*Q0 carriers was 47%, 30% and 23%, respectively. In the 70-79 years group a percentage of 78%, 11%, and 11% was found that is the proportion of both C4A*Q0 and C4B*Q0 carriers was dramatically dropped (p<0.001). The carrier frequency of 8.1 haplotype as defined by carriership of C4A*Q0, TNF2, and Hsp70-2 G was found to be 8.3%, 11.8, and 0% in the three age-groups (p=0.015). This decrease was restricted to the females.

Our results confirm that the C4B*Q0 genotype is a negative selection factor for survival, and indicate that the ever-smoking-related 8.1AH is also associated with decreased life expectancy.

P1093. Maternal influenza during pregnancy and risk of congenital abnormalities

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The teratogenic effect of influenza viruses is currently being debated, therefore we examined the large population-based data set of the Hungarian Case-Control Surveillance of Congenital Abnormalities (1980-1996) to study the possible association between maternal influenza and congenital abnormalities. We evaluated 22843 newborns or fetuses with different congenital abnormalities and 38151 matched controls without any birth defects. The prevalence of maternal influenza during the entire pregnancy and in the second and/or third month of pregnancy was compared between the study groups and crude and adjusted odds ratios (OR) with 95 percent confidence intervals (CI) were determined in conditional logistic regression models. The use of antifever drugs was an effect modifier, therefore the analyses were repeated with the variable of interest stratified. We found a higher prevalence of maternal influenza during the second and/or third month of pregnancy for the group of newborns with cleft lip ± palate (adjusted OR, 3.2; 95% CI, 2.0-5.3), neural-tube defects (adjusted OR, 1.9; 95% CI, 1.1-3.3) and cardiovascular malformations (adjusted OR, 1.7; 95% CI, 1.3-2.3). However, a direct teratogenic effect from influenza

viruses appears to be unlikely, and our results suggest that the higher prevalence of some congenital abnormalities can be explained mainly by fever, because the risk was reduced by the use of antifever drugs. Thus, it is important to consider preventing the potential teratogenic effect of maternal influenza by vaccination and periconceptional folic acid supplementation and by starting adequate antifever therapy as soon as possible after the diagnosis of maternal influenza during pregnancy.

P1094. Medication use during pregnancy in Hungary in recent years

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The purpose of our analysis was to evaluate the medication use during pregnancy among mothers of cases with birth defects and healthy controls in recent time period.
We used the population-based dataset of the Hungarian Case-Control Surveillance of Congenital Abnormalities in order to calculate the frequencies of drug intake by mothers during their pregnancy from 1997 through 2002. Cases with birth defects (n = 7,559) were identified from the HCAR, two healthy control babies (n = 14,448) were identified by public health nurses. For statistical analysis, we applied logistic regression.
Medication use among younger pregnant women (<25 years) was higher compared to older maternal age group (>=25 years) (OR=1.21;95% CI=1.12-1.32). Vitamins, mineral supplements were used by the majority of mothers among both cases and controls (76% any time during pregnancy; 38% during the first trimester). Therapeutic drug use during pregnancy was higher (OR=1.56;95% CI=1.47-1.65) among case mothers (49%) compared to control mothers (38%). Medication use was lower among both case (19%) and control (13%) mothers during the first trimester in comparison with intake during the second and third trimester (28%; 23%, respectively). Our preliminary analysis showed that large proportion of pregnant women use medications in Hungary, which is higher among mothers of cases with birth defects compared to the general Hungarian pregnant population represented by mothers of healthy control babies in the dataset. Our findings suggest the continuous need of postmarketing drug surveillance among pregnant women in Hungary to evaluate the risk-benefit of medication use.

P1095. Distribution of connexin 26 gene and delta(GJB6-D13S1830) mutations in 601 subjects according to their geographical origin

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Molecular testing for mutations in the gene encoding Connexin 26 (*GJB2*) has become the standard of care for genetic diagnosis and counseling of autosomal recessive non-syndromic hearing impairment (ARNSHI).
Epidemiological data collected to date indicate that there is a great diversity in the prevalence of each genetic subtype of ARNSHI in different populations. In most of the studies, a single allele predominate, in particular 35delG in Caucasians, 167 delT in Askhkenazi Jews; 235 delC in Japanese; R143W in Ghana and W24X in India. A few other alleles have a moderate frequency.
Identifying the most frequent mutations leading to NSHI in a given population may contribute to develop molecular diagnostic protocols well suited for that population, with few specific tests capable of detecting most of mutant alleles.
We report data on a cohort of 601 individuals suffering from hearing impairment collected from all over Italy.
Sequencing of the complete Connexin 26 gene coding region and deletion analysis of the common Δ (GJB6-D13S1830) mutation led us to identify 169 unrelated individuals with mutations in Connexin 26 and/or Δ (GJB6-D13S1830):102 patients (60.3%) exhibited a 35delG homozygous genotype, 47 (27.8%) were compound heterozygous 35delG/not 35delG while 16 patients (9.5%) were compound heterozygous not 35delG and 4 showed a dominant mutation.
Our results will give indications about the genetic profile of hearing loss due to mutations in GJB2 and Δ (GJB6-D13S1830) among different Italian areas and will allow to compare on a regional basis the Connexin 26 and Δ (GJB6-D13S1830) gene mutation rates.

P1096. Descriptive epidemiology of Cornelia de Lange syndrome

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CdLS is a multiple congenital anomaly/mental retardation syndrome consisting of characteristic dysmorphic features, microcephaly, hypertrichosis, upper limb defects, growth retardation, developmental delay and a variety of associated major malformations. We present results of the population-based epidemiological study of the classical form of Cornelia de Lange Syndrome (CdLS).The data were provided by 33 registries form 16 European countries. Registries participate in the large European network of birth defect registries - EUROCAT, using the same epidemiological methodologies. 106 cases of CdLS have been identified in the total of 8 604 049 births, which corresponds to a prevalence of 1.23/100000 births. There were 96 live births, 3 stillbirths, and 6 terminations of pregnancy. Prenatal diagnosis by ultrasound examination accounts for 23.5% of all diagnosed cases. Live born infants with CdLS have a high first week survival rate (81%). The most frequent major congenital malformations associated with CdLS are limb defects (73.1 %), congenital heart defects (45.6 %), central nervous system malformations (40.2%) and cleft palate (21.7%). In the majority of cases the karyotype is normal. Identified abnormal karyotypes (46,XYdel(3)(q12q21),inv(5)(p13q13) and 46,XX t(X;22)(p11;qter)) presumably disrupt genes identified to be responsible for CdLS. Maternal age and paternal age do not seem to be the risk factors for CdLS. Almost 80% of the cases born after the 37th week of gestation weight less than 2500 g; low birth weight correlates with a more severe phenotype, including severe limb anomalies. We found no evidence of exposure to consistent teratogenes.

P1097. Investigation of CYP2C9 Polymorphism in six Different Iranian populations

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The Cytochrome P450 (CYP) 2C9 enzyme is involved in drug metabolism and in detoxification of carcinogenic compounds. It is described the polymorphism at colon 144 of the CYP2C9 gene (Cys/Arg) and susceptibility of several types of cancer. Also, it is reported that CYP2C9 polymorphism is involved in drug resistance. To investigate the Cyp2C9 codon 144 polymorphism among different ethnicity, we collected samples from healthy population from six different populations from Iran. The CYP2C9 Cys144Arg genotypes were determined by polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) and direct DNA sequencing analysis in 205 healthy controls.
Among the healthy subjects with Mazandarani, Turkoman, Kord, Turk and Fars (including Shiraz & Yazd) ethnicity, the genotype frequency of CYP2C9 Cys144Arg were 83.7%, 90.2%, 86.6% 92.5% and 83.5% (86% for Shiraz & 81% for Yazd populations) for Arg allele.
No significance difference in CYP2C9 allele distribution was observed between Mazandarani, Turkoman, Kord, Turk and Fars (including Shiraz & Yazd) healthy individuals. In each groups, the distribution of genotypes fits the Hardy-Weinberg equilibrium. We want to use our data to detect the possible association of the CYP2C9 polymorphisms with susceptibility to several types of cancer from Iran.This work was supported by NIGEB project number 197.

P1098. Novel polymorphisms in exon 3 and intron 2 for *CYP2C9* gene

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The Cytochrome P450 2C9 gene has a function in detoxification of carcinogenic compounds. It is described the polymorphisms of the CYP2C9 gene. Also, it is reported that CYP2C9 polymorphism is involved in drug resistance. To investigate the Cyp2C9 polymorphisms among Iranian popupaltions, we collected samples from healthy population. The CYP2C9 polymorphisms were determined by direct DNA sequencing analysis in 11 healthy controls and DU-145 cell line.Among the healthy and patient subjects, we found two novel polymorphisms at codons 112 (ATT→ATC) and 119 (AAA→AGA) in exon 3, also four novel polymorphisms in intron 2, at nucleotides 6117 (C→T), 6108 (T→A), 6109 (A→G) and two base deletion (AG) at nucleotides 6102 and 6103. This work was supported by NIGEB projects number 197.

P1099. Predicting individual metabolizer status using data-mining tools: application to *CYP2D6* data.

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Cytochrome P450 2D6 (CYP2D6) plays a crucial role in the metabolism of xenobiotics and processes about 20% of all commonly prescribed drugs. Genetic polymorphism at the *CYP2D6* gene locus is responsible for pronounced interindividual and interethnic differences in the catalytic activity of the enzyme. Four main metabolic phenotypes have been described in human populations : poor, intermediate, rapid, and ultra-rapid metabolizers. Determination of individual metabolizer status before initiation of therapy is crucial to ensure optimal dosing recommendations; this should improve patient outcome by reducing adverse effects and improving drug efficacy.
The objective of this study is to identify the most informative *CYP2D6* genetic markers for the prediction of individual metabolizer status. To address this issue, several data-mining tools were explored : decision trees, random forests, artificial neural networks, and the multifactor dimensionality reduction (MDR) method. Marker selection was performed in eight population samples of various ethnic origin.
Our results show that the number of polymorphisms required to predict CYP2D6 metabolic phenotype with a high predictive accuracy can be dramatically reduced owing to the strong haplotype block structure observed at *CYP2D6*. MDR and neural networks provided nearly identical results and performed the best. The results of this study will be helpful for the design of time- and cost-effective genotyping tests, adapted to specific populations, that could be used as routine tools in clinical practice.

P1100. European CF survey: results of a European concerted action on the identity and frequency of CFTR gene mutations among Turkish and North African CF patients in Europe

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Background: It is well known that mutation spectra of the CFTR gene vary between populations, even within Europe. Knowledge of these mutation spectra is needed for diagnostic purposes, for counselling in CF families and for screening, either neonatal to improve prognosis, or preconceptional and prenatal to provide for reproductive options. There is only limited knowledge about the mutation spectra in migrant populations in Europe.
Objectives: To determine the identity and frequency of mutations found in Turkish and North African CF patients and to study whether the test-sensitivity of common CFTR gene mutation panels is appropriate for Mediterranean people when offering CF-carrier screening.
Methods: In a survey among 373 European CF-centres, we asked whether and which mutations have been found among Turkish and North African CF-patients.
Results: Fifty different mutations had been found on 75.2% (95%CI: 70.4-80.0%) of the CFTR alleles of patients (n=156) with both parents from Turkey or North-Africa. The mean sensitivity of common CF-gene mutation panels to detect these mutations was 50.6% (95% CI: 45.0-56.2%), and differed significantly between Turkish and North African people: 41.7% (95% CI: 34.7-48.6%) versus 66.4% (95% CI: 57.7-75.2%). A sensitivity of 63.6% (95% CI: 58.2-69.0) can be achieved by expanding the mutation panels with these Mediterranean mutations.
Conclusion: A low test-sensitivity of common CF-gene mutation panels for CF-carrier screening of Mediterranean people was observed. This raises questions on whether and how to implement CF-carrier-screening in a multi-ethnic society.

P1101. Genetic analysis of CFTR mutations in cystic fibrosis patients from Romania

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Objectives: The aim of this study was to improve the number of cystic fibrosis mutations detected in patients from the National Center of Cystic Fibrosis from Timisoara (NCCFT).
Material and methods: the study included a retrospective part which consisted of analyzing the genetic tests results for 79 patients already investigated in collaboration with Royal Manchester Children's Hospital - Genetic Unit (UK) and an original, prospective part in which we selected 17 patients for genetic analysis, based on clinical findings and sweat test. 29 mutations were investigated using a Elucigene™ CF29 kit which detects point mutations or small deletions in deoxyribonucleic acid (DNA) using a method based on ARMS allele specific amplification technology. DNA was extracted from lymphocytes from peripheral blood samples, genomic DNA was amplified by PCR and the PCR products were visualized on a UV transiluminator after electrophoresis on agarose gel and staining with ethidium bromide.
Results: we identified 18 mutated alleles from a total number of 34 alleles and three mutations: ΔF508, G542X and I148T. We combined the new, original data with data from the retrospective part and we obtained new estimates of the frequency for CF mutations in Romania.
Conclusion: the most frequent mutation in western and central Europe, ΔF508 (70%) has a lower frequency in Romania (47,92%). There are still many mutations that remain unidentified (34 %) by investigating only the usual mutations. The great number of mutations and polymorphisms identified up to date (25) reflects the genetic heterogeneity of Romanian population.

P1102. Population study at two additional STR loci D2S1338 and D19S433 in the representative sample of multinational Bosnia and Herzegovina residents

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In our previous population studies of B&H human population, we have used 15 STR loci included in the *PowerPlex 16® System*, twelve Y-chromosomal short tandem repeats loci incorporated in the *PowerPlex® Y System* and 28 Y-chromosome NRY bi-allelic markers. All obtained results were included in Bosnian referent database. In order of future development of this database we have decide to analyze two additional STR loci: D2S1338 and D19S433. Therefore, we have tested 110 unrelated healthy individuals born in the Bosnia and Herzegovina, from three main ethnical groups. Qiagen Dnaeasy™ Tissue Kit was used for DNA extraction from buccal swabs and blood stains, QuantiBlot® assay for quantification and AmpFlSTR®Identifiler® for amplification and detection. Amplification was carried out as described previously. The total volume of each reaction was 12.5µl. The PCR amplifications have been carried out in PE GeneAmp PCR-System 9700 Thermal Cycler according to the manufacturer's recommendations. Electrophoresis of the amplification products was preformed on an ABI PRISM 3100. Numerical allele designations of the profiles were obtained by processing with GeneScan® 3.7.1 and Genotyper® 3.7. Deviation from

Hardy-Weinberg equilibrium, observed and expected heterozygosity, power of discrimination and exclusion were calculated. Also**,** **w**e have compared B&H data with data obtained from geographically closer (neighboring) European populations. Results of this study are going to be used as guidelines in additional investigation of genetic relationship between recent B&H and neighboring populations, originated in our previous studies on Y-chromosome bi-allelic markers, as well in its application in the field of population, human and forensic genetics.

P1103. Detection of deletions/null alleles in small pedigrees

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Null alleles for PCR-based genetic markers can either be due to polymorphisms in the primer annealing sites or to deletions. When analysing diseases with a complex inheritance, the detection of deletions is very important, since deletions may in many cases directly contribute in the causation of disease. Null alleles, other than deletions, are also of interest since undetected null alleles may cause errors in haplotype inference and population genetic analyses. We have earlier described how deletions causing a dominant disease can be detected (Johansson et al. 2005 Hum.Hered. 60:26-35). Here we investigate a method that is useful for complex diseases, but also for normal variation. Null alleles can be detected as a special case of non-Mendelian inheritance, i.e. when a parent and child appear to be homozygous for different alleles. The probability for this event was derived for: a parent and one child, a trio, a trio with one grandparent and a trio with two grandparents. The power to detect a deletion for a fixed overall number of investigated individuals was calculated for SNPs and multiallelic markers with varying allele frequencies. The results show that for SNPs, a trio with two grandparents are always more efficient than the other family types despite a lower total number of founder chromosomes. For multiallelic markers the outcome varies. The method was applied to SNP data in 41 three-generation pedigrees containing 4-5 individuals. Several cases of segregating deletions and other null alleles were detected and will be described.

P1104. The genetic demographic structure of the rural populations in Tomsk region.

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We analyzed the data of the marriage records over the period from 1999 to 2004, and then we calculated the some rates of population structure in our region. Today over 116 nationalities live in Tomsk region. The total population is about 1.057 million including 453 thousands of rural population. The middle age was 26.84±2.13 for men and 24.32±1.55 for women who got married during this period. The part of postreproductive marriages was 10.3%. The part of young men and women (20 year and early) among spouses were 11.4% and 28.3% respectively. The national structure of the married couples included 75 nationalities: 67 for men and 59 for women. About 91.4% Russians, 2.34% Germans and 1.6% Ukrainians were determined among contracting marriages persons. The part of marriages between spouses the same nationality was 83.3%. The ethnic marriage assortativeness (A') was 7.01% for Germans, 11.79% for Russians and 6.38% for Ukrainians. There were about 77% natives of Tomsk region among all marriage couples. The natives of Siberian region averaged 7.7%. The migration rate was 0.409 and did not differ significantly between sexes.
In that way, women get married on 2.5 year earlier than men and twice more often in young age. The dominate nationality in Tomsk region are Russians. The some nationalities are characterized by high rates of ethnical endogamy. The migration activity is low in population of our region.

P1105. Genetic variability revealed by RAPD-PCR in newborn and adult mouse different tissues

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The DNA rearrangements in various organs and tissues of animals have not been studied up to now by PCR-fingerprinting. The aim of present study is revealing of possible DNA polymorphisms by RAPD-PCR in cells of different histogenesis in newborn and adult C57Black/6 and outbreed mice. The brain, liver, skeletal muscle, heart, intestine, lungs, kidneys, spleen, and testicle were used.
The patterns of the total genetic variability of C57Black/6 and outbreed mice had some differences. The general quantity of DNA variations was in several times more (from 2 to 6 times) in outbreed mice than in C57Black/6 mice in two examined periods. However both groups of mice showed the more of the polymorphisms in newborn mice than in adult mice. The individual differences did not reveal in the newborn mice, but at the same time they were very strong in adult mice.
The most genetic variability was revealed in DNA of heart, skeletal muscle and lungs of newborn mice. But in adult C57Black/6 mice DNA polymorphisms of tissues were differed from strong decreasing (skeletal muscle, brain) or complete absence (intestine, heart, lungs, liver, kidneys, spleen) DNA variations. In outbreed mice in contrary we found out the polymorphisms in different tissues.
Our data have shown that the number of genomic alterations rose during ontogenesis and that this DNA variability markedly differed in individual animals.

P1106. Trends in the prevalence of livebirth Down syndrome during 25 years

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Down syndrome(DS) constitutes 8% of cases of registered congenital anomalies in Europe.The objectives of this study were to examine trends in the live birth prevalence of
DS during 25 years in a well defined population in the light of trends in maternal age and prenatal diagnosis.
The material for this study came from multiple sources on births and terminations of pregnancy (TOP)after prenatal diagnosis of DS in 334,305 consecutive pregnancies of known outcome.The study period was divided into 3 subgroups 1979-1988, 1989-1996 and 1997-2003. In the area under study prenatal diagnosis of DS is offered to all women >38 years.Maternal serum screening(triple test)is offered to all pregnant women since 1997 and fetal ultrasonographic scanning is routine practice.
Between 1979-1988,1989-1996 and 1997-2002 TOP for DS was 16.2%, 46.8% and 73.4%, respectively.During the 3 time periods the livebirth prevalence per 10,000 of DS was 9.79, 10.26, and 5.14, respectively.The total prevalence of Down syndrome was 11.69,19.31 and 19.29, respectively.The livebirth prevalence of DS has since 1997 increasingly diverged from the rising total prevalence.The main reason for these observations is the increase in maternal age, from 24.8 to 30.1.
In conclusion the rise of maternal age has brought with it an increase in the number of pregnancies affected by DS.The widespread practice of routine prenatal diagnosis and TOP has counteract the effect of maternal age in its effect on livebirth prevalence of DS. However the high total prevalence of fetal DS shows that more efforts on the primary prevention of chromosomal abnormalities are needed.

P1107. Human X-chromosomal lineages in the Armenian population

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Armenians belong to the one of the oldest Middle Eastern civilizations with a recorded history of about 3000 years. Studies of their genetic diversity were previously carried out at the level of Y-chromosome and mitochondrial DNA. Here we are extending them to a polymorphic segment of the dystrophin locus in the chromosome Xp21.3.
We genotyped thirty-six polymorphisms (substitutions, small insertions/deletions and T-repeat microsatellite) in eight population groups from Armenia and derived the underlying haplotypes. 416 Armenian chromosomes were compared with previously typed 1121 chromosomes from Africa, Asia and Europe. Armenian populations were found to share 42 common haplotypes with other populations. We also found 15 new Armenian specific haplotypes representing 4.6% chromosomes, which can be derived from the frequent haplotypes

assuming simple recombination event.
Our results show that geographically distinct Armenian regions do not significantly differ both in the haplotype composition and in the haplotype diversity (h=0.8) implying that gene flow took place in the whole territory of Armenia. Fst analysis indicate that only 0.23% of genetic variation is among populations and 99.77% - within populations. Likewise, comparable haplotype diversity of Armenian population to Europe and Arabian Peninsula also suggests gene flow rather than isolation as well as a possibility of admixture, consistent with the geography of Armenia, situated at the borders of Europe and Asia. These conclusions are also in agreement with the principal component analysis. These results will be discussed in the context of other genetic systems studied earlier, providing interesting insight into genetic history of Armenian population.

P1108. Cytogenetic study on the effect of *Prestige* oil on human health

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In November, 2002, the oil tanker *Prestige* broke up in front of Galicia, spilling more than 73,000 tons of crude oil. The oil was composed basically of volatile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAH) and resins. From the beginning, a lot of people worked in the cleaning tasks. Among them, there were volunteers that collaborated for one week (V), hired manual workers (MW) and high-pressure cleaner workers (HPW). The aim of this work was to evaluate the genotoxic risk that oil exposure could exert on the human health. Environmental exposure to VOC was evaluated by passive dosimetres and a chromatographic methodology. Two genotoxicity tests were applied on human lymphocytes: micronucleus (MN) and sister-chromatid exchanges (SCE). It is well known that some intrinsic factors may modulate the interindividual different responses to an exposure. Since epoxide-hydrolase (encoded by EPHX1 gene) is one of the main enzymes that take part in COV and PAH metabolisms, and paying attention to the activating character of this enzyme when dealing with these substances, we have also evaluated the association between two polymorphisms in EPHX1 gene and the cytogenetic parameters analysed.
No exposure effect was detected on MN frequency, but HPW showed significantly higher SCE values than controls. Sex effect was observed in SCE test and age influence was significant in the exposed population for both tests. Smoking increased SCE frequency both in exposed and in controls. Finally, the effect of epoxide-hydrolase expected activity on the genotoxicity of the Prestige oil seemed not to be relevant.

P1109. The epidemiology of hereditary diseases in Khakasia Republic

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The medical genetic study of the population of Khakasia Republic (Russia) was performed. Khakasia Republic is situated in the centre of South Siberia, the total population is 542.7 thousands people (the urban population - 336 thousands, the rural population - 206.7 thousands). The most numerous ethnic groups are Russians (79.5%) and Khakass (11.1%), the other groups amount 9.4%. The 286 patients from 200 families with monogenic hereditary diseases were revealed. The 202 patients from 145 families were Russians, 74 patients from 47 families were Khakass. The 8 families with 10 patients were other nationalities (Armenians, Germans and Moldavians). Thus, the 192 patients from 130 families were found with autosomal dominant diseases; the 65 patients from 52 families had autosomal recessive pathology; the 19 patients from 10 families were revealed with X-linked forms. The group of hereditary syndromes predominated in the structure of hereditary pathology (autosomal dominant - 44.5%, autosomal recessive - 42.8%, X-linked - 57.1%). The group of skeletal and connective tissue disorders was more often among autosomal dominant pathology (33.3%). The inborn metabolism defects and neurological pathology (25% and 17.8% respectively) were the most prevalent among autosomal recessive diseases. Previously, we performed medical genetic study in different regions of Siberia (Tomsk region, Altai, Tuva and Yakutia Republics), but we identified rare syndromes such as Lesch-Nyhan, Werner, Diamond Blackfan, Bloch-Sulzberger and LEOPARD syndromes only in Khakasia. We continue the study of load and the prevalence of hereditary pathology in Khakasia Republic.

P1110. ADD3 (gamma adducin) haplotype variation and positive selection

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Adducin functions within the cytoskeleton as an heterodimer that is formed by an a_subunit and either a b_or g subunit. Each subunit is coded by genes (ADD1, ADD2, ADD3) mapping on different chromosomes. In kidney tissue adducin expression leads to a prevalent presence of a-g_heterodimer. Hypertensive patients carrying the ADD1-460Trp variant (rs4961) have a flattened slope of the pressure-natriuresis curve. Epistatic interaction between ADD1-460Trp and ADD3-IVS11+386G (rs3731566-SNP5) polymorphisms affects 24h ambulatory blood pressure, pulse-pressure and pressure-natriuresis relationship in three indipendent studies.
AIM: We investigated the pattern of variation at ADD3 locus starting from SNP5 performing two different approaches: 1) SNP and haplotype analysis; 2) comparative genomic analysis in different populations.
METHODS: Bioinformatic searching in various databases (dbSNP, ABI-Celera, CNG, HapMap, Ensembl, UCSC); genotyping of 100 hypertensive patients; haplotype analysis using the criteria of Gabriel in Haploview package.
RESULTS: 1-ADD3 SNP5 is peculiar of Europeans and the derived G allele (MAF 0,40) is only included in the most frequent and long-range haplotype class (H1). 2- Only in Europeans ADD3 genomic region, but not surrounding genes, reports a negative Tajima's D value. 3-ADD3 HapMap data comparison indicates an unusual frequency spectrum of higher degree of rare variants only in Europeans.
CONCLUSIONS: All these peculiarities raise the possibility that positive selection recently has been acting or has acted in ADD3 region and that the SNP5/haplotypeH1 spread rapidly in Europe, such as in skin pigmentation regulatory genes.

P1111. The CACA *GAS6* haplotype is associated with atherothrombotic stroke

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Growth arrest-specific 6 gene product (*GAS6*) is a tyrosine kinase receptor ligand with anti-apoptotic and pro-thrombotic effects. In a previous study we identified different *GAS6* SNPs and found an association between c.834+7G>A, in intron 8, and stroke. The purpose of the present study was to analyze the association between specific *GAS6* haplotypes and disease in a population of 656 stroke patients (457 ischemic and 199 hemorrhagic) and 150 healthy controls.
Genotyping was performed by use of different PCR-based methods. The THESIAS program was used to measure linkage disequilibrium and haplotype frequencies.
Results: Genotype and haplotype analysis revealed that the c.834+7AA genotype was found associated with protection from stroke (OR: 0.53; 95% CI: 0.32-0.89). After adjustment for known vascular risk factors, this association was maintained for the atherothrombosis-related (atherosclerotic, lacunar and deep ICH) strokes and it was even stronger when the c.834+7A allele was present in a specific haplotype (CACA) of four *GAS6* polymorphisms (c.280+170C>G, c.712+26G>A, c.713-155T>C, c.834+7G>A). The prevalence of the CACA haplotype was 9.7%, 11.8% and 14.6%, respectively in patients with ischemic stroke, hemorrhagic stroke and in controls. After adjustment for known vascular risk factors, the CACA haplotype was independently associated with ischemic stroke (OR 0.48 [0.28-0.83]), as well as with atherothrombotic strokes (OR 0.43 [0.24-0.79]).
Conclusions: The AA genotype of the *GAS6* c.834+7 G>A SNP and more strongly the *GAS6* CACA haplotype are associated with protection from ischemic and, atherothrombosis-related strokes.
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P1112. Study of genetic diversity among 18 Iranian Ethnic group using Y chromosome marker

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Since the Y chromosome passes from father to son, man's paternal ancestry can be traced using the DNA on his Y chromosome (Y_DNA). This is a useful tool for study the genetic diversity among different ethnic groups and also the migration patterns in different geographical region.

In this study we aim to look at 27 marker on the Y chromosome (15 Y-STR and 12 Y-SNP) for investigation of genetic diversity among different Iranian ethnic groups (farsis, kurde ,Lors ,Balooch , Azari, Sistany Turkaman, Arab, Negro, Bandari,Gilak, Mazandarani, kermani, Yazdi,Korasani, Jews, Ashoori, Armenian).

Blood samples from unrelated (at least three generation) individuals from each ethnic group were collected (Total=1370). DNA was extracted from blood sample using standard protocols. Multiplex PCR systems have been set up for the simultaneous PCR amplification of many of these markers.

The present study is an attempt to determine the genetic variation among different population in our country for application in evolutionary studies, forensics, medical genetics and genealogical reconstruction.

P1113. Evidence of a major gene related to the infection of *Leishmania braziliensis* in a western Amazonian Population.

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American cutaneous leishmaniasis (ACL) is a parasitic disease caused by several species of the protozoa *Leishmania* (subgenera *Viannia* and *Leishmania*) and is endemic from Yucatán to Northern Argentina. A sample of 313 individuals from Monte Negro (10º15'S, 63º18'W), a small rural county in the State of Rondônia, Brazil, was collected in a large Genetic-Epidemiologic survey and subjected to the Montenegro skin test (MST), aiming to uncover genetic mechanisms involved in the human host response to ACL. In this region of Amazon, ACL is mainly caused by *Leishmania (Viannia) braziliensis*. The prevalence of positive reactions was 0.42. Complex segregation analysis was applied to the MST data corrected for age and sex, using the unified model of Lalouel *et al.* (1983). As shown bellow, the analysis indicated the presence of a major recessive gene (frequency $q = 0.548$) that influences the outcome of MST in the studied population.

Complex Segregation Analysis of the Montenegro skin test in Monte Negro, Rondônia, Brazil

Model	*-2 ln L*	χ^2	P	test	e.p.	AIC
1.Mixed	428.91				4	436.911
2.Sporadic	455.94	27.024	0.000	2vs.1	0	455.936
3.No major gene	455.14	26.233	0.000	3vs.1	1	457.144
4.No multifactorial	428.98	0.070	0.791	4vs.1	3	434.982
5.Recessive (d=0)	***428.92***	***0.005***	***0.946***	***5vs.1***	***3***	***434.916***
6.Additive (d=0.5)	449.52	20.611	0.000	6vs.1	3	455.523
7.Dominant (d=1)	452.98	24.072	0.000	7vs.1	3	458.983

-2 ln L = minus twice the log likelihood. e.p. = estimated parameters
AIC = Akaike's information criterion

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P1114. Determination of the Carrier Frequency of the Common GJB2 (Connexin-26) 35delG Mutation in the Greek Cypriot Population

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Mutations in the GJB2 (connexin 26) gene are responsible for more than half of all cases of prelingual recessive inherited non-syndromic deafness in Europe. One specific mutation 35delG, accounts for up to 70 % of the mutations detected in European populations and is one of the most frequent disease mutations identified so far. The aim of this study is to determine the percentage of carriers of this mutation in the Greek Cypriot population.

Genomic DNA was isolated from a total of 405 healthy unrelated Greek Cypriot adults. Screening for the frameshift 35delG mutation was performed by using an allele-specific PCR protocol. Moreover, using the Poisson probability distribution, we compared the carrier frequencies of the 35delG mutation of the Greek Cypriot population to the various European and Middle Eastern populations.

The carrier frequency in the Greek Cypriot population was estimated to be 2.5 % and is similar to that observed in other European populations. The variance estimate for 35delG mutation produces slightly wider intervals with the Poisson model when compared with Binomial probability variance estimate.

P1115. Distribution of Y-chromosome minimal haplotypes in Ukraine

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Y-chromosomal microsatellite are highly valuable in human evolutionary and human history populations studies. Nine Y-chromosome-specific human microsatellite loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385a/b) were typed in 243 males with Slavic origin from Ukraine with the use of fluorescent PCR followed by high-throughput fragment analysis on a single-wavelength automated DNA sequencer. The distribution and frequencies of minimal haplotypes were determined and compared with other populations based upon data from the Y-chromosomal Haplotype Reference Database (YHRD). Nine Y-linked STR-loci were found to generate 190 different haplotypes, out of which 162 (85%) were unique. The mean of haplotype diversity in Ukrainian population was 0.992. Locus diversity values ranged from 0.29 (DYS392) to 0.82 (DYS385a/b). It was shown that the several ancestral neighbour's haplotypes (16/13-31/24/11/11/13/14.15, 16/13-30/25/11/11/13/11.14 and 16/13-30/25/11/11/13/11.15) took part in formation of Ukranian male line.

P1116. Single origin for a worldwide common Hirschsprung (HSCR) susceptibility non-coding *RET* mutation

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HSCR is a complex genetic disease characterized by colonic aganglionosis. The *RET* proto-oncogene is a major HSCR gene, whose coding sequence mutations account for a small proportion of cases, while a common noncoding variant in intron 1 has been proposed as a major disease factor.

We have collected HSCR families from six countries over three continents, and genotyped 2,672 individuals for 14 markers at the *RET* locus. Overall, the T allele of the intron1 "I1.357" SNP, demonstrated to be an enhancer mutation, showed the highest transmission to affected sibs (τ=0.85; p=5.06 *10^{-66}), with a frequency of 59% among transmitted alleles and 24% in untransmitted chromosomes. Differences among sample sets were consistent with the higher prevalence of HSCR in Chinese than in Caucasian populations. To prove the identity by descent

of the mutation, we reconstructed transmitted and untransmitted haplotypes. In Caucasians, the two haplotypes that included the I1.357 T allele, called “long” and “short”, were the most frequently transmitted, while in the Chinese sample only the long haplotype was present. These haplotypes share a common allelic combination extending from 5’ UTR to exon 5.
We conclude that the enhancer mutation arose on the long haplotype which, after the Asian-European split, rearranged to give also the short haplotype. Accordingly, a recombination hotspot between introns 5 and 8 was estimated by the PHASE software and confirmed by linkage disequilibrium analysis.
These findings represent a first step to elucidate the genetic history that has led to the current worldwide HSCR associated *RET* alleles distribution.

P1117. Hitoh Syndrome

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The male of 9 years old, first child in the family the other two are females children and are normal. Products of full term and forceps delivery. Doubtful cephalheamatoma is found to be normal. Father and sister are not clever. Clinical findings reveal mild mental retardation, intelligent quotient is of 50. Mild webbing of neck, knock -knee noted, hypothenar and thenar eminenent in both hands shows wasting. His gait is disturbed due to shortening of one of lower limb. Right testes palpable in inguinal region with testes not palpable in the left scrotal sac or inguinal area. Ultrasound of abdomen with testes not visualized with its appearance in the inguinal region. Audiogram showed mild to moderate hearing loss.
The above case study is observed and the cytogenetic investigations carried out, the karyotype of subject and both the parents found to be normal and none of the paternal and maternal fore fathers are with such abnormalities and the no complications documented during the delivery and at the time of carrying the child. The lower intelligent quotients and levels are not answered by chromosomal analysis of any syndromes of known karyotype anomalies.
So we anticipate that further molecular analysis would answer the queries of this investigation.

P1118. Mixed modeling and multiple imputation to characterize epistasis in association studies with unobservable phase

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Background: The recent explosion of molecular level information coupled with large epidemiological studies, presents an exciting opportunity to uncover the genetic underpinnings of complex diseases; however, analytic methods accounting for (1) synergy across a large number of genetic loci and environmental factors and (2) uncertainty in allelic phase, will be essential. Methods: To this aim, we propose a combination of multiple-imputation and mixed effects modeling to determine whether combinations of genetic polymorphisms contribute to ART associated hypertriglyceridemia in N=626 individuals with HIV-1. Four candidate genes were considered: ApoC-III, SREBP1c, TNF-alpha and MDR-1. Simulation studies were performed to characterize the power and false discovery rates associated with this method. Results: The proposed analytical technique identified an overall interaction between ApoC-III, TNF-alpha, and PI exposure within Hispanics but not in Caucasians and Blacks. Simulation studies suggested that a sample size of N=340 provides greater than 80% power to detect a standard deviation in multi-locus effects as low as 0.4 times the error standard deviation in 1,2,3 and 4-locus genotype models. False discovery rates were consistently <10% for 1,2,3 and 4-locus models. Conclusions: The pathophysiology of dyslipidemia in ART-treated HIV patients is likely to be multifactorial and involve synergy among several gene and gene-drug pathways. This manuscript presents a novel statistical method to explore potential multi-locus effects on plasma TGs across candidate genes that accounts for potential ambiguity in allelic phase. Ultimately, this approach will allow investigators to discover complex clinical and biological associations in the context of unobservable genetic information.

P1119. Low density microarrays for HLA-DQA1 genotyping.

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The aim of this work was to develop an oligonucleotide microarray for the analysis of HLA-DQA1 locus. At present, 28 alleles of HLA-DQA1 are known. The developed microarray allows to analyze 9 groups of alleles of the HLA-DQA1. The group 1 includes 0101, 0104, 0105, 0107 alleles; the group 2 includes 0102 alleles; the group 3 includes 0103 allele; the group 4 includes 0106 allele; the group 5 includes 0201 allele, the group 6 includes 0303 alleles; the group 7 includes 04 alleles; the group 8 includes 05 alleles, the group 9 includes 06 alleles. The procedure engages two-stage PCR with fluorescently labeled primers and hybridization with oligonucleotide microarray. The fluorescent signal is analyzed using a chip-reader equipped with CCD-camera. The sensitivity of the method is about 10-100 pg per analysis. The microarray was tested with DNA samples isolated from fresh blood, saliva and spot of blood. To confirm the results of genotyping the selected samples were also sequenced and/or analyzed by allele specific PCR-kit (“DNA-Technology”, Moscow, Russia). Totally, we tested 55 samples of DNA of individuals from the Moscow region. The following alleles frequencies have been found: for the group 1 - 16,9%; group 2 - 16,2%, group 3 - 3.8%, group 4 - 0%, group 5 - 9,2%, group 6 - 9,2%, group 7 - 13,8%, group 8 - 34,6%, group 9 - 0,8%. This microarray is suggested to be used in forensic DNA analysis, medical analysis of histo-compatibility, association studies of the HLA-DAQ1 locus with different diseases.

P1120. Distribution of ancestry informative markers in Russian populations

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Individual ancestry can be revealed using a set of ancestry informative markers (AIMs). These markers may be useful to control for the presence of genetic structure due to admixture (Bonilla et al. 2005). In the present study, AIMs were represented by mitochondrial DNA (mtDNA), Y-chromosome and three SNPs in the P-gene associated with human pigmentation. Twelve Russian populations from European Russia were studied. It was found that 1.5% of mtDNAs found in Russians fall into East Eurasian haplogroups. However, two populations from North-Western Russia (Velikij Novgorod and Volot) exhibited more than 3.5% of East Eurasian lineages. Y-chromosome data show that only Pskov population (also from North-Western Russia) demonstrates a significant similarity with Finno-Ugric/Baltic populations. Variability of Y-chromosome microsatellite loci indicates that high frequency of haplogroup N3 in Pskov population (35%) is caused mostly by the presence of Baltic N3-variants, although gene pools of Russians from central and southern parts of European Russia are characterized by prevalence of lineages characteristic for Finno-Ugric populations. Results of the P-gene variation study show that there are significant differences between distribution of SNP*R419G in Russian populations. Frequency of 419G allele (which is thought to be associated with green/hazel colour of eyes) is much higher in North-Western Russians (6% in Novgorod and Pskov samples) than in South-Western ones (3.6% in Belgorod sample). Thus, the data presented demonstrate that North-Western Russian populations represent a complex pattern of population structure, reflecting diverse interactions that occurred at different times between populations of West and East Eurasian ancestry.

P1121. Three distinct methylation profiles in human H19 DMR with conserved monoallelic expression

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About 0.5 % of human genes are imprinted with a parent-of-origin specific expression of one allele related to differential methylation of specific CpG-rich regions (DMRs).
The IGF2/H19 locus is the most widely studied imprinted region particularly in mice. State of the art in methylation analysis consists of bisulphite modification of genomic DNA that converts unmethylated

cytosines in uraciles while leaving methylated Cytosine unchanged, and subsequent cloning and Sanger sequencing which is cumbersome
We took advantage of a new real time quantitative sequencing technology, Pyrosequencing®, to analyse the methylation profiles in two IGF2 and the H19 DMR in human lymphocytes and placentas.
In the IGF2 DMR, we found an average methylation level compatible with the expected median-methylated status, but with a highly variable methylation level at successive CpGs, yielding a DMR-specific profile irrespective of the biological sample.
In contrast the methylation profile often departed from the expected median-methylated stage in maternal lymphocytes as well as in placental samples in the H19 DMR. Rather, three distinct methylation levels were observed that were specific for a certain individual. Moreover, a predominantly monoallelic expression of H19 was preserved independently of the DMR methylation status demonstrating that DMR allele-specific methylation and allele-specific expression of imprinted genes are not always correlated.
Preliminary analysis of relatives from three families showed that the inheritance of these methylation profiles was compatible with a simple Mendelian model considering one gene with three alleles.
These results should lead us to be cautious when transferring results derived in mice to human epigenetics.

P1122. A-to-C exchange in 3'-utr of the human *IL12B* gene is associated with Th1-mediated infectious diseases

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IL-12 is a key cytokine of Th1-mediated cellular immunity against infectious diseases. It is comprised of p19 and p40 subunits encoded by two distinct genes, *IL12A* and *IL12B*, respectively. p40 subunit is shared by another Th1 cytokine, IL-23, which is also actively involved in anti-infectious immunity. Rare mutations in *IL12B* gene cause atypical disseminated infection due to poorly virulent mycobacteria and salmonella. A-to-C exchange was described in 3'-utr of the gene in position 1188 that correlates with decreased protein secretion and might affect common predisposition to Th1-mediated infectious diseases. We performed association study of *IL12B* 1188A/C polymorphism with active tuberculosis (TB) and salmonellosis in Russians from Tomsk city. Three hundreds and four TB, 49 salmonellosis, and 129 control cases matched by age and gender were genotyped by *IL12B* 1188A/C polymorphism. Also, 40 family trios with tuberculosis affected offspring were studied by Transmission/Disequlibrium Test (TDT). The prevalence of the 1188C allele in TB (0.240±0.018) and in salmonellosis (0.347±0.048) was significantly higher than in controls (0.174±0.024; χ^2 = 4.075, p=0.044 and χ^2 = 11.264, p=0.001, respectively). Also, the 1188C allele was overtransmitted from heterozygous parents to TB offspring as compared to the alternate (13 vs. 2 times; TDT = 8.067, p = 0.005). Thus, both case-control and family-based association data suggest that *IL12B* 1188A/C polymorphism predispose to Th1-mediated infectious disorders due to intracellular bacteria. This association may be because of impaired expression rate of the 1188C allele or due to its linkage disequilibrium with other common polymorphisms in *IL12B*.

P1123. The Val Borbera Project: epidemiological and genealogical analysis of an isolated population in Northern Italy.

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The Val Borbera is an isolated valley in the Appenine Region in Northern Italy that has been geographically isolated from the surrounding area until recent years. The population was mainly farming until 50 years ago and they seem to have had a constant but limited growth over the centuries until a large portion emigrated to the Americas at the beginning of the last century. The part of the population that did not emigrate is still living in the valley or in the surroundings and can be traced easily. It should correspond to about 2000 people. The Val Borbera Project is aimed at the establishment of a comprehensive collection of biological samples and clinical records in which to study the genetic basis of common diseases and of quantitative traits. A medical questionnaire and a set of laboratory analysis were proposed to the population and most of the inhabitants were willing to participate. The availability of demographic data from the city and the church archives will be exploited to trace back genealogical trees and to reconstruct the familial structure of the population. Results of the first set of clinical and genealogical analysis regarding about half of the population will be presented.

P1124. SNP fine mapping of genes and flanking sequences. Way to find regulatory elements

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The complexity of higher organisms is -more than previously thought- attributable to the evolution of regulatory elements. Alternative promoters have recently been shown to be common to almost half of all human genes. The function of regulatory elements can be modulated by alleleic variants usually termed "regulatory SNPs". These may affect transcription levels, alternative splicing and mRNA stability as well as translation or post-translational modification.
In a candidate gene based LD-mapping project of the QT-interval, we discovered a SNP (rs727957 G>T) located 50 kb upstream of the KCNE1 gene which had an effect on QT interval in the general population (KORA S-4000, p =0.0051).[1]
HapMAp data indicates this SNP may be under selection (polymorphic in CEU but monomorphic in HCB, JPT and YRI populations). Bioinformatic analysis shows that the transversion can produce a new transcription factor binding site for the muscular transcription factor TEF-1. Analysis of cDNAs from 48 different human hearts showed that 3 alternative promoters of KCNE1 exist. All identified transcripts of KCNE1 (contain up to 5 exons) are translated to the same KCNE1 protein. SNP rs727957 influences alternative promoter usage of KCNE1.
High resolution LD-mapping of complex traits thus is shown to be a powerful gene annotation method for functional genomics and systems biology.

P1125. Lactase persistence in Sardinian villages (Italy).

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The ability to digest the milk sugar lactose as an adult (lactose persistence) is a variable genetic trait in human populations. The lactase-persistence phenotype frequencies vary in human populations, generally low frequencies may be found in the majority of tested populations in sub Saharan Africa, but, in some populations, particularly pastoral groups, it is significantly more frequent. In Europe, the highest frequencies of lactase persistence can be observed in the north western populations, where milk-dependent pastoralism was developed very early, traversing towards the south and east there is a decrease in prevalence of lactose persistence. Recently, the T allele of a C/T polymorphism in a potential regulatory site located 13,910 bp upstream the lactase gene was found to be completely associated with lactase persistence in Northern Europeans. A significant, although less strong association, was also observed with a second -22,018 bp G/A mutation.
Our aim, is to perform for the first time a molecular screening of lactose tolerance distributions, by testing two biallelic markers (-13910 C>T and -22018 G>A) apparently associated with lactose tolerance, in Sardinian populations (Italy) with different types of economy. In particular we have chosen two villages in the Oristano region (Central Sardinia), one village (Cabras) is situated at the sea level, where prevails fishing economy, and the other (Scanomontiferro) is situated at an altitude of 400m above sea level where pastoral activity is prevalent.

P1126. What is the best way to break large genealogies for the genetic study of complex traits?

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Large genealogies provide many individuals for linkage analysis. However, the software available for nonparametric multipoint linkage analysis is limited in the complexity of the families it can handle. Partition into subfamilies is necessary.

Two methods have been proposed to partition pedigrees automatically, one based on factor analysis[1], and the other on a maximum-clique partitioning approach[2]. Both were proposed for the study of quantitative traits.

We adapted these methods to qualitative trait analysis and assessed the sensitivity of subsequent NPL analysis to these approaches and their parameters. Better results were obtained when the pair relationship measure on which the breaking is based is highly correlated with the linkage informativity of the pair. Additionally, results were appreciably improved if partitioning was first based on affected individuals only.

Our results are illustrated with a genome-wide NPL analysis of hypertension in a 2636-member pedigree from the isolated village of Campora, South Italy. We show that the genome-wide significant linkage detection of a new locus on 8q22-23 as well as the replication of two known loci (1q42-43 and 4p16) are conditioned on the quality of the genealogy partition.

Our results illustrate the great sensitivity of linkage analyses in large genealogies to the quality of the genealogy partition. They also show that, partitioned in an optimal way, large genealogies from population isolates offer a good power to detect genetic risk factors for complex diseases.

1 Pankratz and Iturria (2001) Genet Epidemiol ; 21 Suppl 1:S258-63
2 Falchi et al (2004) Am J Hum Genet ; 75(6):1015-31

P1127. Use of isolated population to understand genetic bases of late onset progressive hearing impairments

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Hearing impairment is a frequent disorder affecting one in 1,000 children, whose prevalence increases with age. At least 60% of cases are due to genetic causes; and may manifest itself as syndromic (20% of all cases) or non-syndromic (80% of all cases) form. Despite the fact that a number of loci has been identified for hearing loss, a poor knowledge exist on the genetic bases of late onset progressive hearing impairments (starting around the age of 40), the most common cause of adult auditory deficiency, resulting from yet unidentified interactions between environmental and strong genetic factors. The study of late-onset forms in isolated populations is particularly useful because of both genetic and environmental homogeneity. We have selected 3 isolated Italian villages for which genotypes and audiological phenotypes have been collected: Campora (600 inhabitants), Carlantino (1400) and Stoccaredo (400). In Campora 51% of the whole population has been diagnosed as suffering from late onset severe hearing impairments. In Carlantino 54% of the whole population is affected by bilateral sensorineural hearing loss (10% in people aged less than 40). Data from Stoccaredo are still under evaluation. A first known locus between markers D1S104 and D1S466 (DFNA7) has been already identified in Campora. Since a huge number of information has been collected for each individual (biochemical, instrumental and lifestyle data) it would be much easier to understand the relationships between genes involved in hearing loss in these populations, including modifier genes, and to define the interplay role between genetic and environmental factors.

P1128. Familial risk for migraine with aura and migraine without aura among first-degree relatives of migraineurs

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Migraine is a primary, chronic, headache, affecting 11%-20% of the general population. Familial clustering of migraine with aura (MA) and without aura (MO), the two most common subtypes, was described in several studies.

Probands and their first-degree relatives were classified according to their migraine subtype, to evaluate familial risk for MA and MO. The use of family history to classify relatives as migraine sufferers was also validated; though probands were able to correctly identify their affected relatives, migraine was still underestimated: only relatives with a direct interview were thus included.

Familial risk was estimated using the relative risk (RR) in first-degree relatives. Prevalence in our general population has been estimated previously at 1.4% for MA, and 6.0% for MO. The risk for MA and MO was evaluated separately in 51 families of probands with MA and in 94 families of migraineurs with MO.

The RR of MA and MO among first-degree relatives of probands with MA was 10.58 (95% CI: 6.24-17.93) and 6.51 (95% CI: 4.98-8.51), respectively. The RR of MO of first-degree relatives of probands with MO was 8.82 (7.10-10.95). The risk for MA in relatives of MO probands was not possible to estimate, as only a few parents were affected by MA. Our findings show that, in our sample, MA is less frequent than MO as previously described. The RR for first-degree relatives of migraineurs with MA is highly suggestive of a genetic contribution. For MO, the results also point out to familial clustering.

P1129. Mitochondrial DNA polymorphism in Evenks and Buryats from Chita region of Russia

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Chita region is administrative unit of Russian Federation situated in the Transbaikal Region, north to Mongolia and China. Now most of the population are Russians who started to arrive there since the 17th Century. Indigenous peoples of the region are Evenks living in the north of the region (about 1000) and Buryats in the south (about 42000). We have studied mtDNA polymorphism (by HVS-I sequencing and high-resolution RFLP) in 81 Evenks and 115 Buryats from the area. In total, 33 and 75 different HVS-I haplotypes were found in Evenks and Buryats, respectively. The most frequent haplogroups in Evenks were C and D; A, G, N9 and Z haplogroups were also present. Buryats are more heterogeneous, with predominance of C, D, and G, while A, B, F, Z and M7 were found to be less frequent.. West-Eurasian derived haplogroups (H, J, U and a few others) encompassed about 8% of samples in Evenks and 11% in Buryats. Interestingly, one Buryat individual carrying haplogroup X was identified. The two Buryats with 16325 substitution in haplogroup C and two Evenks encompassing 16111 change in haplogroup A may mark Native American founder haplotypes. The two ethnic samples had only 8 mtDNA haplotypes in common, indicating quite isolated gene pools of these populations. In contrast to that, studied by us Buryats had 23 lineages in common with Buryats from the Repubic of Buryatia (Derenko et al. 2003). The study was supported by RFBR grant 04-04-48792.

P1130. Manganese superoxide dismutase (MnSOD) polymorphism and breast cancer risk

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Oxidative damage induced by the generation of reactive oxygen species (ROS) by exogenous and endogenous exposures is believed to be implicated in breast cancer. MnSOD is the primary antioxidant enzyme in the mitochondria with a function of the detoxification of superoxide free radicals and thus protecting cells from oxidative stress. MnSOD is a nuclear-encoded protein that is transported into the mitochondrium through the mitochondrial targeting sequence. A polymorphism in this sequence with a valine to alanine substitution is believed to alter transport of the enzyme into mitochondria, has been

reported to be associated with increased risk for breast cancer among women with low intake of dietary antioxidants.
We examined what role of MnSOD polymorphism plays in the development of breast cancer in a case control study. MnSOD genotype was determined in 156 sporadic premenopausal breast cancer patients and 228 healthy controls using a PCR-RFLP method. Statistical analysis of the data suggested that there was no allelic association between the cases and controls (Chi2=3.447, df=2, P=0.178). Whereas the AV genotype gave an odds ratio of 1.476. (OR=1.476, 95%CI=0.978-2.229, df=1, P=0.063). However it was statistically insignificant. The frequency of the A allele was 54.17% in cases and 55.26% in controls. The frequency of the V alalle was 45.83% in cases and 44.74% in controls. In conclusion, MnSOD polymorphism might be a weak genetic risk factor for breast cancer in premenopausal sporadic breast cancer women in Turkey

P1131. Defining the allelic variants of *NAT2* gene in the Moscow population using biochips

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The product of the gene *NAT2* (N-acetyltransferase 2) participates in detoxication of some arylamine derivatives (in particular 2-aminophtorene, 4- aminobiphenyl and - naphftylamine), which are strong mutagenes and cancerogenes, and metabolizes various drugs. More than 17 SNP and 36 alleles of *NAT2* gene are known, which code fast (R/R, R/S) and slow (SS) acetylation mode: *4, *11 (A, B), *12 (A, B, C, D), *13, *18 and *5 (A, B, C, D, E, F, G, H, I, J), *6 (A, B, C, D, E), *7 (A, B), *10, *14 (And, B, C, D, E, F, G), *17, *19. We have developed the biochip for the analysis of all 17 SNP of gene. The developed biochip allows simultaneous analysis of the 17 SNP within a *NAT2* gene fragment approximately 900 b.p. in length. The 86 healthy persons of the Moscow population were investigated. It was found that 47% of individuals had the slow acetylator genotype. These data are in accordance with previously described frequency of slow acetylators in other European populations (40-60%).
The work has been particular supported by a grant „Fundamental Science for Medicine“ from the Presidium of the Russian Academy of Sciences.

P1132. Online Encyclopedia for Genetic Epidemiology studies (OEGE): www.oege.org

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The Online Encyclopedia for Genetic Epidemiology studies (www.oege.org) aims to collate information and links about and for human genetic research studies relevant to population genetics and health. It gives annotated links to the world's population and case/family genetic epidemiological studies and relevant gene and sequence databases, genetic variation databases, trait measurement, resource labs, journals, software, general information, disease genes and genetic diversity. A links page to genetic and epidemiological societies and meetings is available and links pages to relevant commercial suppliers of genotyping, phenotyping and software tools and services will be made available. Other original OEGE content includes online software tools (e.g. a Hardy-Weinberg equilibrium test calculator and a sequence manipulator) and tutorials are also being added. It is designed to help professionals working in this or associated fields, acting as a World Wide Web portal for Genetic Epidemiology studies, but it may also be of interest to genealogists and historians interested in molecular genealogy. Visitors can submit new information by web form or email, there are gene discussion forums; and update and other news is available by RSS feed, home page news or email.Initiated January 2006. Online now. Please email any enquiries or comments to webmaster@oege.org

P1133. Investigation of the genetic basis of oral-facial clefting in humans

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Oral clefts (OCs) are important to public health in terms of birth prevalence, costs of care, and psychosocial impact. Recent genetic and epidemiological studies have led to new theories about the causes of cleft lip and/or cleft palate (CL/CP).
It has been shown that there is considerable international variation in the frequency of OCs influenced by numerous factors. There are many parts of the world - including Eastern Europe - for which we still have little or no information on the frequency of OCs. Out of 382 newborns with OCs registered in Lithuania during last 5 years there were 99 (25.6%) syndromic and 283 (74.1%) non-syndromic CL/CP cases. The incidence of OCs in Lithuania was 1.84 for 1000 newborns.
A subset of candidate genes (*TGFA*, *END1*, *RARA*, *TGFB*, *SK1*, *DLX1/2*, *PITXZ*, *PAX9*, *AP2*, *TTF2*, *PVRL1*) have been shown to play an important role in the development of the head, with particular relevance to the development of the lip and palate. Additional genes coding for growth, signalling and transcription factors that play a role in the facial development include *JAG1*, *SHH*, *PTCH*, *CREB1*, *GLI3*, *FGFR1*, *CASK*, *TCOF1*, *FGFR2*, *DLX5/6*, and *PAX3*. The purpose of our research was to test the hypothesis that *TGFA*, *TGFB3*, *GABRB3*, *RARA*, *BCL3* and *IRF6* genes are involved in the etiology of CL/CP.
Three chromosomal rearrangements identified by comparative genomic hybridization in 3 out of 27 syndromic CL/CP patients from Lithuania point to 14q32.11-qter, 20p13-pter and 21q21.2-qter as potential chromosomal regions to search for new candidate genes.

P1134. Parental smoking and polymorphisms in epoxide hydrolase and glutathione S-transferase P1 affect nonsyndromic orofacial cleft risk in offspring

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Background: In addition to genetic predispositions, environmental factors, such as parental smoking, are thought to be involved in the pathogenesis of nonsyndromic orofacial clefts. The toxicity of smoking during embryogenesis varies in time, dose and frequency of exposure, and is dependent on maternal metabolism, transfer of the toxins from the extraembryonic tissues to the embryo and the detoxification capacity of the individual. Genetic variations in the detoxification enzymes microsomal epoxide hydrolase (*EPHX*, exon 3, exon 4), glutathione S-transferase-P1-1 (*GSTP1*, exon 5) may therefore affect the teratogenicity of lifestyles such as smoking. We investigated the role of polymorphisms in *EPHX* and *GSTP1*, parental periconceptional smoking, and their interaction with cleft lip with or without cleft palate (CLP) risk in the offspring.
Methods: Dutch Caucasian non-consanguineous triads (mother, father and child) with complete valid genetic data per polymorphism were included (*EPHX* exon 3: 118 CLP and 70 control triads, *EPHX* exon 4: 149 CLP and 95 control triads, *GSTP1* exon 5: 69 CLP and 95 control triads). Transmission disequilibrium testing (TDT) and logistic regression analyses were performed.
Results: Polymorphic *EPHX* exon 3 and exon 4 alleles associated with CLP (TDT; $p \leq 0.05$). Paternal *EPHX* exon 4 polymorphisms and paternal smoking increased CLP risk, OR: 2.5 (95% CI=1.1, 5.9). Homozygosity for *GSTP1* exon 5 polymorphisms in mothers or children approximately 3-fold increased CLP risk.
Conclusion: The *EPHX* exon 3 and exon 4, *GSTP1* exon 5 polymorphisms, and *EPHX* exon 4 polymorphism in combination with paternal smoking contribute to CLP risk.

P1135. Islet amyloid polypeptide (amylin) haplotypes and bone mineral density in young and elderly women in Southern Sweden

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BACKGROUND AND AIM: Islet amyloid polypeptide (IAPP or amylin) is a candidate hormone which is predominantly expressed by the pancreatic beta-cells and co-secreted with insulin in response to food intake. Several studies have implied a role for IAPP in bone remodeling. For instance, IAPP knockout mice display increased numbers of osteoclasts and develop an osteporosis-like phenotype in adulthood. In the present work we have investigated whether different IAPP haplotypes are associated with bone mineral density (BMD) in young women at peak bone mass and elderly women at high risk of fracture. MATERIALS AND METHODS: 1005 young women from the Malmö Peak-study (age 25±0.1 yrs, BMI 23.0±3.7 kg/cm2) and 1044 elderly women (Malmö OPRA-study; age 75±0.1 yrs, BMI 26.2±4.2 kg/cm2) were recruited. The primary phenotype was BMD assessed by DXA. Body composition data, calcaneus ultrasound estimates and fracture data (OPRA-study) were also available. Short nucleotide polymorphisms (SNPs) in the vicinity of the IAPP gene were retrieved from the International HapMap Genotype Database and genotyped by PCR using the ABI SNP genotyping assay. RESULTS AND DISCUSSION: Obtained data and an update of this study will be presented.

P1136. Analysis of polymorphisms of *VDR*, *COL1A1*, *ESR1* and *DBP* genes in three ethnic groups of Volga-Ural region.

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Osteoporosis (OP) is a complex disease with a strong genetic component. There are significant differences in osteoporosis morbidity between different ethnic groups. The polymorphisms of four candidate genes for osteoporosis (*Fok*I- *VDR, Sp*I-*COL1A1, Pvu*II, *Xba*I- *ESR1, (TAAA)n-DBP)* were studied. We analyzed genotypes and alleles frequency distribution of these polymorphisms in three ethnic groups of Volga-Ural region (116 Bashkirs, 85 Russians *and* 95 Tatars). We found genotype frequency distribution of *Fok*I, *Xba*I and *(TAAA)n* polymorphisms in Bashkirs ethnic group differed from that in Russians and Tatars. The frequency of *VDR*f* allele was 28.3% in Bashkirs versus 44.5% in Tatars (χ^2=12.8, p=0.0003, df=1) and 40.6% in Russians (χ^2=6.48, p=0.01, df=1) and the frequency of *VDR*f*f* genotype was 6.6%, 17.9% (χ^2=15.06, p=0.0005, df=21) and 17.6% (χ^2=7.06, p=0.029, df=2), respectively. The frequency of *ESR1*x*x* genotype was 6.8% in Bashkirs versus 21.3% (χ^2=7.61, p]=0.022, df=2) in Russians and 43,9% in Tatars (χ^2=39.18, p]=0.00001, df=2). The frequencies of *GC-18*8*, *GC-18*10*, *GC-18*11*, *GC-18*12* alleles of *DBP (TAAA)n* polymorphism were 16%, 79%, 5%, 0% in Bashkirs, 5.5%, 86.2%, 7,7%, 1% in Russians (χ^2=13.72, p=0.0033, df=3) and 4%, 88%, 6%, 2% in Tatars (χ^2=21.21, p=0.00009, df=3), respectively. No significant differences in frequencies of alleles of *Sp*I and *Pvu*II polymorphisms were observed. Our data showed that frequencies of these polymorphisms of *VDR*, *ESR1, DBP* genes are significant different between ethnic groups. Because of specific ethnic allele's frequency distribution, these polymorphisms reflect population's differences of osteoporosis morbidity. Future studies of OP need to take into account ethnic factors.

P1137. Research of STR polymorphism of PAH gene in Kazakhstan

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We investigated the frequencies distribution of highly polymorphic microsatellite TCTA-repeats located in 3 intron of PAH gene (STR allele). For research we have taken DNA from blood of 201 not related representatives of the Kazakh nation. In the Kazakh population we have revealed 8 various alleles. This alleles distinguished from each other on 4 b.p. We have found 27 genotypes, most often was a STR*244/*248, (0,144). There was no deviation in distribution of frequencies of a STR-genotypes from Hardy-Weinberg equilibrium. In the Kazakh populations allele STR*244 (frequency 0,310) is most often as well as in other populations of the world. Allele STR*248 was on the second place by the frequency (0,220). On the data of the literature this allele most frequently meets at Turkish peoples of Volga-Ural region in Russia (from 0,13±0,03 at Chuvashs to 0,18±0,02 at Bashkirs) [Akhmetova V., 2001].

We have done the comparative analysis of distribution of frequencies STR allele of a gene PAH and have found out authentic distinctions between sample of the Kazakhs and Udmurts(chi-square=14,180, p=0,041), Maris (chi-square=14,780, p=0,029), Mordvins (chi-square=19,60, p=0,004) and Chinese (chi-square=19,130, p=0,005) [Goltsov et al., 1994; Akhmetova V., 2001]. Observed heterozygosity was 0,810. This meaning was higher than in populations of Europe (0,800), Asia (0,730) and Volga-Ural region of Russia (0,790).

P1138. Inactivation status of *PCDH11X*: sexual dimorphism in gene expression levels in brain

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Genes escaping X-inactivation are predicted to contribute to differences in gene dosage between the sexes and are the prime candidates for being involved in the phenotype observed in individuals with X chromosome aneuploidies. Of particular interest is *ProtocadherinX* (*PCDH11X* or *PCDHX*), a recently described gene expressed in brain. Although *PCDH11X* has a homologue on the Y chromosome in humans and is predicted to escape from X-inactivation, there was only weak evidence for the escapee status of this gene, from experiments in lymphoblastoid cell lines.

Employing bisulfite sequencing we analyzed two CpG islands in the 5´end of the gene and found absence of methylation on both the active and the inactive X chromosomes, in blood leukocytes and in brain tissue (grey mater), giving a strong indication that *PCDH11X* escapes inactivation in humans. Furthermore, a sexual dimorphism in levels of expression in brain tissue was observed for this gene, by quantitative real-time PCR, with females presenting an up to twofold excess in the abundance of *PCDH11X* transcripts. We relate these findings to sexually dimorphic traits in the human brain.

P1139. The analysis of the STR polymorphism FGA, vWF genes and D3S1358 locus in East Eurasian populations

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STR loci are used for personal identification in the medical and forensic casework because of their multiallelic variation and, consequently, high level of informativeness. There are substantial differences in allele frequency distributions for independence DNA loci among ethnic groups. Therefore in each region and for each ethnic group is essential to create one's population-genetic base on allele and genotype frequencies for DNA loci used in the world-wide forensic-genetic practice. We have studied the STR polymorphism of FGA, vWF genes and D3S1358 in ten East Eurasian populations: Bashkirs, Tatars, Chuvashes, Komies, Mordvinians, Udmurts, Russians, Ukrainians, Belarusians and Yakuts. The exact test demonstrated that STR of the FGA gene had deviations from Hardy-Weinberg equilibrium in Udmurts and Russians ($p<0.05$) only. Comparison of the informativeness of STR loci FGA, vWF and D3S1358 allows us to consider the first more informative by findings of statistical parameters of forensic importance: the average value of the observed heterozygosity was 0.831 in FGA, 0.818 in vWF and 0.762 in D3S1358; polymorphism information content (PIC) and power of discrimination (pD) were also higher in FGA (PIC=0.830, pD=0.949) than in vWF (PIC=0.765, pD=0.915) and in D3S1358 (PIC=0.721, pD=0.895); matching probability (pM) - 0.051, 0.085 and 0.011, consequently; the coefficient of allelic variety was 6.662 in FGA, 4.892 in vWF and 4.230 in D3S1358; index of Shennon diversity - 0.344, 0.321 and 0.285, consequently. In conclusion, investigated FGA, vWF and D3S1358 loci can serve as highly informative markers for genetic research, forensic casework and determination of biological relatedness of individuals.

P1140. Angiotensinogen M235T polymorphism and the risk of myocardial infarction and stroke among hypertensive patients on ACE-inhibitors or β-blockers

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Introduction: It remains difficult to predict the effect of an particular antihypertensive drug in an individual patient and pharmacogenetics might optimise this. Angiotensinogen is a component of the renin-angiotensin system and ACE-inhibitors and β-blockers both have a direct influence on this system.

Objective: To investigate whether the association between use of ACE-inhibitors or β-blockers and the risk of stroke or myocardial infarction (MI) is modified by the T-allele of the angiotensinogen M235T polymorphism.

Methods: Data were used from the Rotterdam Study, a population-based prospective cohort study in the Netherlands. In total, 4093 subjects with hypertension were included from July 1st, 1991 onwards. Follow-up ended at the diagnosis of MI or stroke, death, or the end of the study period (January 1st, 2002). The drug-gene interaction and the risk of MI or stroke was determined with a Cox proportional hazard model with adjustment for each drug class as time-dependent covariates.

Results: The interaction between current use of ACE-inhibitors and the angiotensinogen M235T polymorphism was multiplicative on the risk of MI (interaction HR:4.00; 95%CI:1.32-12.11). There was a non-significant increased risk of stroke (interaction HR: 1.83; 95%CI:0.95-3.54) in subjects with the MT or TT genotype compared to the MM genotype. No interaction was found between current use of β-blockers and the AGT M235T polymorphism on the risk of MI (HR:1.30; 95%CI:0.60-2.83) or stroke (HR:1.39; 95%CI:0.81-2.39).

Conclusion: Subjects with at least one copy of the 235T allele of the AGT gene might have less benefit from ACE-inhibitor therapy.

P1141. CYP2C9 polymorphism in patients under phenytoin therapy

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The hepatic enzymes CYP2C9 and CYP2C19 are responsible for the metabolism of numerous clinically important drugs such as phenytoin with a narrow therapeutic index. Phenytoin is mainly oxidized by CYP2C9 and to a minor extend by CYP2C19. Polymorphism of CYP2C9 and CYP2C19 has been reported previously. Pharmacogenetic polymorphisms, can divide the population into four phenotypes including poor metabolisers(PM), intermediary metabolizers (IM), extensive metabolisers (EM) and ultrarapid metabolisers (UM). In our study, the frequency of CYP2C9*2 and CYP2C9*3 allelic variants were examined in a group of 76 Turkish epileptic patients who received phenytoin for at least two weeks 200-500 mg/day orally. Genomic DNA was isolated from peripheral leukocytes using phenol-chloroform extraction procedure.

The allelic variants were studied by polimerase chain reaction and restriction fragment length polymorphism. Plasma phenytoin concentrations were determined utilizing fluorescence polarization immuno assay (FPIA) on Abbott AxSYM system and high performance liquid chromatography (HPLC). The frequencies of CYP2C9 genotypes in the study group were 75%, 17,1%, 8% for CYP2C9*1/1, CYP2C9*1/2 and CYP2C9*1/3 respectively. The mean phenytoin serum concentrations were determined to be 8.32 µg/ml for genotype CYP2C9*1/1, 10.93 µg/ml for CYP2C9*1/2 and 17.43 µg/ml for CYP2C9*1/3. The results show that there is a strong correlation between CYP2C9 genotypes and phenytoin dose requirement. It is suggested that the CYP2C9 genotyping can be used routinely to obtain efficient phenytoin therapy and to lower the risk of concentration dependent intoxications of phenytoin in mutated carriers.

P1142. Predictive genetic testing in obstetrics and gynecological pathology

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The review of on-going studies dealing with molecular analysis of genetic polymorphisms involved in some common obstetrics and gynecological diseases and their complications in fetal development. The study included endometriosis, adenomyosis, habitual (recurrent) miscarriages, preeclampsia , placental insufficiency. Relevant gene cassettas has been composed for each disease and functionally significant polymorphisms for each gene has been studied. Special emphasis has been paid to the set of metabolic genes, participating in the 1st and the 2nd phases of detoxification processes. Functionally inferior alleles of GSTT1,GST-1, CYP19 and NAT2 genes in the women were found to be non-random associated with endometriosis and adenomyosis origin, progression and treatment efficiency. Genotypes GSTT10/0, GSTM10/0 GSTP1 D/-- and ACE I/I- with early recurrent spontaneous miscarriages and placental insufficiency . Genotypes PAI14 G/4G., eNOS 4a/4a, +14/+14HLA-G genes were associated with preeclampsia.. The predictive testing for these and some other relevant pathology in pregnant and non-pregnant women as well as in newborns (chromosomal disorders, neural tube defects etc.) in conjunction with molecular carrier testing for common monogenic diseases contribute to so called Genetic Form of Reproductive Health suggested and used in our laboratory. Owing to this Form the women at high risk of relevant pathology could be efficiently detected before or early in pregnancy and thus could be subjected to preventive treatment. Special Pharmagen-Biochip which enables efficient identification of 14 polymorphisms in 8 different metabolic genes (CYP1A1,CYP2D 6,GSTM1,GSTT1,NAT2,MTHFR,CYP2C9 and CYP2C19) has been elaborated. Perspectives and limitations of its application in routine genetic polymorphisms testing are discussed.

P1143. Hereditary prosopagnosia as a very common deficit - first worldwide survey

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Prosopagnosia or face blindness refers to a deficit in face recognition in the presence of intact sensory and intellectual function. The acquired form of prosopagnosia is a rare condition and the congenital form was previously considered to be even less common. However, in the German population we recently assessed a very high congenital prosopagnostic prevalence of 2.5 % (CI 95% 1.3 - 3.8). We could further show that this congenital anomaly had almost always familial recurrence which is compatible with simple autosomal dominant inheritance. We therefore introduced the term hereditary prosopagnosia (Kennerknecht et al. 2002, Grüter et al. 2005).

The high frequency of this deficit hitherto only described in the Caucasian population prompted us to extend our search to other ethnic groups in the form of a questionnaire based screening at universities in India, Hong Kong, Zululand, and among Campesinos in the peruvian Altiplano. Candidates suspicious for prosopagnosia underwent a semi-structured interview. The following frequencies of prosopagnosics were found: India 1 prosopagnosic out of 160, Hong Kong 9/534, Zululand 3/573, Peru 9/490. These figures are minimal estimates as not all suspicious candidates could be interviewed. In half of the index probands we could also find two or more affected first-degree relatives supporting an autosomal dominant segregation pattern. The similar prevalence of prosopagnosia in very different populations is suggestive of a very old mutation.

P1144. Genetically isolated population study: molecular basis of taste genetics

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Some individuals are taste blind to bitter compound having the thiourea moiety (-N-C=S), such as phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP). Taste sensitivity to PTC/PROP is bimodally distributed: nontasters make up approximately 30% of the adult caucasian population and tasters make up the remaining 70%. This percentage can vary, depending on geographic location and ethnic origin.

Some evidences suggested that the taster status is a result of the interaction between PTC/PROP gene and enviromental factors.

In this study we determine the correlation between taster status and PTC genotype, and the influence of PROP status and food preferences in Carlantino, a genetically and culturally isolated village located in southern Italy. It was settled at the end of 16th century by a few number of founders. The endogamy rate, calculated during last century, was 99.5%. Actually, Carlantino counts 1519 inhabitans.

A sample of 587 adults, 15 to 89 years of age, was recruited from the village. Analysis of variance was used to assess differences in bitter perception and in liking of food groups as functions of PROP status and food adventurousness.

Our data show that the percentage of PROP status is comparable to caucasian population. Women are more sensitive to PTC/PROP than men, and PTC genotypes explain only the 64% of phenotype. We didn't find any correlation between PROP status and food preferences.

For the first time, these data have been validated by a high number of samples coming from a genetically isolated population.

P1145. RFLP analysis of DNA polymorphisms of pERT87-8/Taq1 and 16intron/Taq1 loci in radiologists and control group.

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Background: One of most important consequence of ionized radiation is appearance of point mutations. There showed effects of ionizing radiation on the chromosomes of embrional and somatic cells, which are lead to chromosomal instability, involved in cancerogenesis, but the role of intragenic molecular markers from non-coding regions remains largely uncertain.

Aims: Carrying out a comparative analysis of restriction fragment's length polymorphism of pERT87-8/Taq1 and 16intron/TaqI loci in group of technicians who are exposed to ionized radiation (during 5-25 years) and in control group.

Materials and Methods: Were studied DNA samples obtained from 53 radiologists (91 X-chromosomes) and from 70 X-chromosomes of control group (CG). The prevalent duration of employment was from 10 to 15 years (26.4%). We used polymerase chain reaction of pERT7-8/TaqI polymorph site and 16intron/TaqI locus with RFLP-analysis. For the validation of results were applied methods of variational statistics, using of X2 Pearson criterion.

Results: E1 allele of 16intron/TaqI locus (without site of restriction) was greatly more frequent in CG compared to radiologists (0.773 v. 0.348); the distribution of haplotypes was significantly different (X2 = 78.3, df = 1, $p < 0.01$). The frequency of allele A1 of pERT87-8/TaqI in radiologists was 2.4-fold higher in radiologists compared to control group (0.451 v. 0.185), and haplotype's distribution were significantly different (X2 = 27,7, df = 1, $p < 0.01$).

Conclusions: Was revealed statistically significant difference of the frequency of polymorphic site pERT87-8/Tag1 and 16intron/Tag1 locus in radiologists compared to healthy donors.

P1146. Pleiotropic effects of genes involved in salt-sensitivity

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Dietary sodium is an important contributor to hypertension. The degree of salt-sensitivity varies among individuals. Genes that play a role in salt-sensitivity are alpha-adducin (*ADD1*) and genes involved in the renin-angiotensin system (RAS). We studied the association between the *ADD1* Gly460Trp polymorphism, angiotensinogen gene (*AGT*) M235T polymorphism and the angiotensin II type 1 receptor (*AT1R*) C573T polymorphism in relation to blood pressure, atherosclerosis, cardiovascular and cerebrovascular disease. This study was part of the Rotterdam Study and Rotterdam Scan Study. We analysed the data with Cox and logistic regression, adjusting for age and sex. For *ADD1*, we found that carriers of the T allele had an significantly increased mean intima-media thickness of the common carotid artery ($p=0.04$), a modest but significantly increased risk of any stroke (HR 1.22, 95 % CI: 1.02-1.45), ischemic stroke (HR 1.29, 95 % CI: 1.02-1.63), hemorrhagic stroke (HR 1.07, 95% CI: 0.59-1.92) and myocardial infarction (HR 1.33, 95 % CI: 1.05-1.69), compared with the GG genotype. The TT genotype of *AGT* significantly increased the mean systolic and diastolic blood pressure ($p=0.03$) and significantly increased the risk of carotid artery plaque (RR 1.25, 95% CI: 1.02-1.52). This genotype also significantly increased the mean volume deep sub cortical white matter lesions ($p=0.008$) compared with the MM genotype. No significant associations were found for *AT1R*. We show that genes involved in salt-sensitivity have pleiotropic effects influencing not only blood pressure, but also several cardiovascular and cerebrovascular outcomes.

P1147. Sex identification in humans by variable-stringency PCR of Y chromosome alpha satellite

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In this report we describe a method for sex identification in humans using Y chromosome specific primers for the centromeric alphoid region. A method called variable-stringency PCR was designed to amplify a specific 281/285 bp product from male DNA, and some random DNA products from both male and female samples, in order to asses the sex of unknown human samples. The amplification of Y chromosome alphoid DNA is a very robust reaction, not very sensitive to the quality and source of the DNA sample. The variable-stringency profile of the amplification reaction consisted in a succession of high-stringency / low-stringency / high-stringency stages, in all cases the stringency being determined by the annealing temperature in the PCR reaction. We used the sex diagnostic method presented here to assign the sex of some unknown human samples, both males and females. The method provided the correct sex in all cases. In conclusion, our study indicates that the co-amplification of both Y chromosome specific and random DNA sequences in a unique reaction mixture is a fast, sensitive and reliable method providing sex identification in humans, thus being very useful in forensic research.

P1148. The genetic structure of S.Tomé: a case-study of human microevolution

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The extent to which human population clusters emerge from underlying genetic patterns or are imposed on data by particular sampling schemes is a fundamental problem in current anthropological and biomedical research. By applying methods for assessing population structure solely on the basis of individual genetic similarity, several studies have shown that individuals sort into clusters that correspond to broad geographic regions. However, much less attention has been dedicated to evaluate how individual variation is structured on a smaller-scale. As a contribution to the understanding of population structure at the microgeographical level, we have studied the genetic patterns of the small plantation island of São Tomé (832 km2) by coupling a transect sampling strategy with a Bayesian clustering approach. Using data from only 15 microsatellite loci typed in 394 unrelated individuals from

14 localities, we found evidence that São Tomé is far from being a single panmitic unit, despite the maximum distance between any two sampled sites being less than 50 km. This uneven distribution is clearly more related to language than to geographic distance and was best captured by two clusters. One of the clusters predominates in villages where the Angolar creole is the major autochthonous language and carries a distinct imprint of genetic drift, indicating that this could have been one of the first maroon communities in the Atlantic slave trade. Our observations demonstrate that neither genetic microdifferentiation is confined to archaic human societies nor homogenization is the only expected outcome of modern periods of population expansion.

P1149. Incidence of Spinal Muscular Atrophy Typel in Tunisia Estimated from Consanguineous marriages.

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Spinal muscular atrophy (SMA) is an autosomal recessive disorder and an incidence in Europe of 1/6,000 - 1/10,000 Incidence can be estimated from rates of first cousin consanguineous marriages using Dahlberg's formula: If both C' and C are determined one can estimate the frequency of allele (q) and therefore of the disease (q^2) according to the following formula derived from the Dahlberg's formula: q = [C (1-C')] / [C (1-C')+16(C'- C)]. In Tunisia, the frequency of consanguineous marriages in the general population has been established . In the present study 52 SMA types I unrelated families were enrolled. . SMA type I diagnosis was confirmed in all cases on the basis the detection of homozygous exon 7 SMN1 gene deletion .

The frequency of first cousin marriages was C' = 42.30 % (22/52). The frequency of consanguineous marriages was 30/52=57.7%. These result show an increase of about 2 fold in consanguinity among parents of SMA type I patients with respect to the general population. Using the first equation given in the introduction q the gene frequency of SMA type I can be calculated to be 0.0343 and a heterozygote frequency (2pq) of 1/15.The incidence (q^2) of SMA type I in Tunisia can be calculated as 1 / 850.

The estimate reported in the present study should be considered as a value of incidence at birth of SMA type I.

In conclusion,. SMA type I is a frequent genetic disorder in Tunisia.

P1150. Molecular genetics and epidemiology of spinocerebellar type 8 ataxia in Spain

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Spinocerebellar ataxias (SCA) are caused by unstable trinucleotide repeat expansions. Sevent genes have been cloned, *SCAs1-3*, *SCAs6-7, SCA12* and *SCA17,* with a CAG repeat which encodes a polyglutamine tract. The exception is SCA8, which consists of an exonic but untranslated CTG repeat. We present here the molecular analysis of 248 unrelated familial and 749 sporadic and idiopathic Spanish cases of SCA. Over the familial cases 8,93% were SCA1; 30,36% SCA2; 34,82% SCA3; 5,36% SCA6; 8,04% SCA7; 10,71% SCA8; and 1,79% SCA17. In SCA8 the CTG range goes from 85 to 183 repeats (109,97% ± 22,13%; Pearson Coef. =20,12%). Maternal transmissions presented elongations of the triplet CTG combined sequence ranging from +2 to +13 repeats (7,5 ± 5,5; Pearson Coef = 73,33%). In contrast, paternal transmissions presented contractions ranging from -1 to -17 repeats (-9,75 ± 5,97; Pearson Coef = -61,23%). Nine giant SCA8 expansions have been detected in unaffected adult individuals and originated from homozygous SCA8 affected mother with alleles of moderate expanded size. In contrast, homozygous males usually transmited 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 frequence 98% and 77 to 86 CTGs with frequence 2%. We have not found any giant allele in this sample. About 60% of familial ADCA cases remained genetically unclassified. No SCA mutations were detected in the 749 isolated and idiopathic cases of spinocerebellar ataxia.

P1151. The transferability of tagSNPs derived from HapMap to an Estonian population

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The recently published Haplotype Map of the human genome shows the patterns of variation in four populations and will help researchers in their quest to find complex disease genes by reducing the number of variants or single nucleotide polymorphisms (SNPs) to test. However, the usefulness of this selection process needs to be verified in populations outside those used for the HapMap project. In this study, we analyzed 1090 individuals from Estonia. The population of this northern European country has been influenced by different migrations from Europe and Russia. We genotyped 1536 randomly selected SNPs from two 500 kb ENCODE regions on chromosome 2. We observe that the tagSNPs selected from the CEU HapMap samples (derived from U.S. residents with northern and western European ancestry) capture most of the variation in the Estonian sample (90-95% of the SNPs with a minor allele frequency >5% have an r2 of at least 0.8 with one of the CEU tagSNPs). Overall, we observed that the sample size, the allelic frequency and the SNP density in the dataset used to select the tags, each have important effects on the tagging performance and have to be taken into account when designing association studies. In order to estimate the relatedness of haplotypes, a median-joining network analysis was performed. The common haplotypes are shared in all studied populations. As expected, the Estonian samples usually share their haplotypes with the CEU samples and there are low frequency haplotypes that are only seen in the HapMap population recruited in Africa.

P1152. Analysis of allelic variation in the three GAS6 receptors' genes, *TYRO3, AXL* and *MERTK* in a Spanish population

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Growth arrest-specific 6 gene (*GAS6*) has anti-apoptotic and proliferative effects through its interaction with TAM (**T**YRO3-**A**XL-**M**ERTK) tyrosine-kinase receptors. We have recently shown an association between *GAS6* polymorphisms and atherothrombotic stroke. As a first step to study association with diseases like atherothrombosis and cancer, we have analysed the variation of TAM genes in our population.

Methods: Twenty-two candidate functional variants in the TAM genes, selected from public databases (dbSNP, Ensembl), were analysed in a minimum of 50 healthy controls by means of PCR, RFLP, SSCPs and sequencing.

Results: One SNP among 8 analysed in *AXL* was polymorphic (frequency>1%) in our population and we found 5 new *AXL* variants: one in 5'UTR and three in intron 19 that were polymorphic and a two nucleotide deletion in intron 1. In *TYRO3*, four of the five selected SNPs were polymorphic and we identified two novel intronic polymorphisms. Seven out of the nine selected variants in *MERTK* were confirmed as polymorphic and two novel SNPs were found in intron 15.

Allelic frequencies of the selected SNPs matched with those reported, excepting two SNPs in *MERTK* and one in *TYRO3* which had an inverted allelic frequency.

Conclusion: Only 55% of the TAM SNPs selected from public databases were polymorphic in our population and three had an inverted allelic frequency than reported. SSCP and sequencing analysis allowed for the identification of nine novel SNPs and a deletion mutation in TAM receptors genes. We thank Spanish MEC (SAF 2001-1059-C02 and SAF 2004-07539-C02) and ISCIII network C03/07) for grants.

P1153. Is tension-type headache inherited?

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Objective. To investigate the importance of genetic and environmental factors in the etiology of tension-type headache with a population-

based twin sample.
Subjects and Methods. Twin pairs were recruited from the population based Danish Twin Registry. A total of 16,181 twin pairs were eligible for the study. They received a posted questionnaire about tension-type headache. Only twin pairs where both twins replied were included.
Results. A total of 3,523 monozygotic (MZ), 4,150 dizygotic (DZ) same gender and 3,526 DZ opposite gender twin pairs were included. The prevalence of tension-type headache was significantly more frequent in men than women. The MX analysis indicates that tension-type headache is caused by a combination of additive genetic effects, common and unique environment effects. The heritability estimates were 48% in men and 44% in women.
Conclusions. Tension-type headache is likely to be slightly influenced by genetic factors.

P1154. Thymidylate synthase gene polymorphisms in Croatian population

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Thymidylate synthase (TS) is crucial enzyme in the nucleotide biosynthetic pathway because it calalyzes the reductive methylation of dUMP by 5,10-MTHFR to form dTMP. TS gene has been an important target for a variety of chemotherapeutics such as 5-FU. The human TS promoter region includes polymorphic enhancer containing two or three 28-bp tandem repeats and has been implicated in affecting on TS mRNA expression. The majority of individual human TS alleles harbor either a double repeat (2R) or a triple repeat (3R) for this polymorphism, creating genotypes of 2R/2R, 2R/3R i 3R/3R. Individuals homozygous for the 3R were found to have elevated intratumoral TS mRNA and protein level. Recently identified G→C SNP in the second repeat of the 3R alleles has shown that the 3R sequence with G has three to four times greater efficiency of translation than the 3R with C and the 2R sequence. Due to associations of the TS polymorphisms with the prognosis of several tumor types, we performed a study to determine the distribution of TS polymorphisms in Croatian population.
A total of 125 healthy unrelated individuals were genotyped for the TS 5‘ UTR polymorphisms using PCR-RFLP method with HaeIII restriction enzyme. Genotype frequencies for 5’ UTR TS polymorphisms were 26,4 %, 16%, 2,4%, 42,4%, 8,8% and 4% for 2R/3G, 3G/3C, 3G/3G, 2R/2R, 2R/3C, 3C/3C genotype respectively.
Our results showed that in Croatian population low TS expression genotypes were more frequent (55.2%) than high TS expression genotypes (44.8%) but not significant.

P1155. TPMT gene polymorphisms in Croatian population

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Thiopurine methyltransferase (TPMT) catalyzes the S-methylation of azathioprine (AZA), 6-mercaptopurine (6-MP) and thioguanine, medications widely used to treat malignancies, rheumatic diseases, dermatologic conditions, inflammatory bowel disease and solid organ transplantat rejection. Low TPMT activity plays a significant role in the occurence of life-threatening myelosupression, a serious toxicity of thiopurine drugs. Altered TPMT activity predominantly results from single nucleotide polymorphisms (SNPs). To date, eight TPMT alleles have been identified, including three alleles (TPMT*2, TPMT*3A and TPMT*3C) which account for 80-95% of intermediate or low enzyme activity. Ten percent of individuals with intermediate activity are heterozygous at the TPMT gene locus and 0.3% are homozygous for low activity alleles.
The aim of our study was to estimate allelic frequency for three SNPs in TPMT gene in the Croatian population. DNAs obtained from 350 unrelated individuals were genotyped for the TPMT*2, TPMT*3A, TPMT*3B and TPMT*3C SNPs using allele-specific PCR or PCR-RFLP method.
The frequency of heterozygous TPMT genotype in Croatian population was 5.7%. The frequency of the three allelic variants of the TPMT gene were: 0.6% for TPMT*2, 4.2% for TPMT*3A, and 0.9% for TPMT*3C. The TPMT*3B allele was not detected in any of the samples analyzed.
In conclusion, this study demonstrate that the TPMT*3A allele is a common allele in the Croatian population. The low frequency of heterozygous TPMT genotype in Croatian population can be explained by interethnic variations of TPMT alleles. In our oppinion this information would be helpful for identifying patients at high risk of inadequate responses to thiopurine therapy.

P1156. Insertion/Deletion DNA Polymorphisms in Rajasthan Tribal Populations and Rajputs

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In recent times, polymorphic DNA markers are widely used to study the genomic diversity of Indian populations as most are selectively neutral, more ubiquitous and have higher heterozygosities than polymorphic protein and enzyme markers. As new alleles are not generated at Alu insertion/deletion loci, and as there is no identified selection pressure on these loci, these loci have gained importance in the study of genetic structures of human populations. In the present study six human - specific insertion/deletion polymorphisms were studied in two endogamous tribal populations, namely, Minas and Bhils of Banswara and Rajputs of Rajasthan. DNA samples from 79 unrelated individuals (37 Bhils, 23 Minas and 19 Rajputs) were analysed. Of these polymorphic markers five are Alu insertion/deletion markers (Alu PV 92, Alu FX III B, Alu D1, Alu APO, Alu ACE and Alu-CD4), while the sixth marker (mt → NUC) pertains to a mitochondrial DNA segment, 540 bp in length, which got inserted into human nuclear genome. The results of this study show that all loci are polymorphic in all three populations. Most of the loci showed high levels of heterozygosity in all three populations. Genetic data allow researchers to determine the relatedness of different racial and ethnic groups and to arrange them in an evolutionary or phylogenetic tree. Special statistical packages will be used for statistical analysis of the data.

P1157. A twin study of ten cardiovascular risk factors

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Aims: Cardiovascular disease itself and several of its risk factors exhibit a significant degree of heredity. The degree of heredity can be quantified as heritability. In this study, we provide a list of ten established cardiovascular risk factors in order to put the genetic contribution levels into a wider cardiovascular perspective. Thus, the purpose of this study was to estimate the heritability of these ten risk factors and compare them.
Methods: Subjects were recruited from an extensive study of MZ twins initiated in the late 1970ies and the early 1980ies. The present series of 155 pairs (68 male pairs and 87 female pairs) were between 38 and 57 years old (mean age 44 years). Spearmans rho was used to check for within-pair correlations in MZ twins.
Results: A list of ten risk factors is presented below.
Conclusion: Several risk factors exhibit significant heritability. Comparing risk factors in the same twin panel gives the opportunity to point out which risk factors are inherited and which are not.
Within-pair correlation coefficients (with 95% confidence intervals) in decreasing order in 155 healthy monozygotic twins for ten risk factors of cardiovascular disease.

Variable	Within-pair correlation
Lp (a) lipoprotein	~1.0 -
Body Mass Index	0.76 (0.68-0.82)
Total cholesterol	0.68 (0.59-0.76)
Systolic BP	0.64 (0.54-0.72)
Homocysteine	0.53 (0.41-0.63)
Diastolic BP	0.51 (0.38-0.62)
Triglycerides	0.46 (0.33-0.58)
CRP	0.40 (0.26-0.52)
NO_x	0.32 (0.17-0.45)
Fibrinogen	0.27 (0.12-0.41)

P1158. Analysis of the UGT1A1 promoter polymorphism in Sao Miguel population (Azores, Portugal)

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A dinucleotide polymorphism in the TATA box promoter of the UDP-glucuronosyl transferase 1 (*UGT1A1*) gene is implicated in Gilbert syndrome (GS), a benign form of unconjugated bilirubinemia. Population studies show that this polymorphism has racial variability, being the (TA)7 allele the most frequently associated to Caucasian patients. Recently, we demonstrated that Sao Miguel population has an admixed genetic background composed mainly of European, Jews and Africans.

To investigate the nature and incidence of the *UGT1A1* mutation in our population, we studied the promoter region in a group of 24 patients with suspected GS and 76 unrelated healthy blood donors. The polymorphism was detected using PCR and fragment analysis by capillary electrophoresis.

Out of 24 suspected GS patients, 17 (70.8%) were homozygous for the most frequent mutation (TA)7/(TA)7, 4 (16.7%) were heterozygous (TA)6/(TA)7 and 3 (12.5%) were homozygous for the normal allele (TA)6/(TA)6. In the control group, we identified 0.7% (TA)5 alleles, 72.4% (TA)6 alleles and 26.9% (TA)7 alleles, and four genotypes: 1.3% (TA)5/(TA)6, 52.6% (TA)6/(TA)6, 38.2% (TA)6/(TA)7 and 7.9% (TA)7/(TA)7. These results show that the frequency of the (TA)7 allele was 0.79% in suspected GS patients and 0.33% in 76 healthy control subjects.

Considering the Azorean genetic ancestry and the presence of the (TA)5 allele, which is characteristic of black populations, we expect to find the (TA)8 allele also present in Africans. For this reason, we are analysing a total of 469 healthy individuals, which are representative of the 6 municipalities of Sao Miguel Island. (paularpacheco@hdes.pt, DRCT founding).

P1159. Genetic variation of the 3' VNTR region of human dopamine transporter gene (DAT1) in the Iranian population

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Variable numbers of tandem repeats have wide application in genetic population study, because of their stability across generation on change in size upon passage to offspring they have been known as valuable tools for genetic study among individual and population. One of them has located with a 40 - bp core unit in the 3' untranslated region of dopamine transporter gene (*DAT1*). *DAT1* gene plays a role in genetic diseases of the brain. This gene acts to transport released dopamine into presynaptic terminals of the brain. Allele distributions of *DAT1* polymorphisms were analyzed in Iranian ethnic groups in order to examine the effect of geographical and linguistic affiliation on the genetic affinities mentioned groups. Amplification of the human dopamine transporter gene was performed by the polymerase chain reaction, 449 Samples were selected to determine polymorphism of the VNTR locus, all samples collected from 8 Ethnic groups including *Pars* (5 regions), *Azeri(Turk)*, *Kurd*, *Gilak*, *Lur*, *Arab,* from Iranian population. Genomic DNAs were extracted from whole blood. Screened 898 chromosome showed four alleles (6, 7, 8, 9, 10 and 11) which distribution of alleles were identical among more different sampling region so that allele10 had high frequency in North, West and Southwest and South while in Center and East Allele 8 was predominant in one ethnicity (Mashhad) and was seen much more in other two ethnicity (Esfahan and Yazd)in comparison with other ethnicities. This study shows DAT1 distribution in Iran has independently gene flow from other studies.

P1160. Gender-specific association of homozygous *VN1R1* pheromone receptor 1a allele genotype in humans

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Pheromones are water-soluble chemicals used as signals that provide information about gender, dominance and reproduction within individuals of the same species, while they elicit profound neuroendocrine and physiological changes. Although multiple functional pheromone receptor genes are present in insects and mammals, there is only one such gene found to be functional in humans, namely the *VN1R1* gene, encoding for a putative seven-transmembrane protein and whose transcripts are found in the human olfactory mucosa. We have undertaken a large mutation screening approach in 125 adult individuals (66 males and 59 females) from 3 population groups (Hellenic, Greek Cypriot and Iranian) to investigate whether the allelic differences, present in the *VN1R1* gene, are gender-specific. Here we show that, although both *VN1R1* 1a and 1b alleles are found in chromosomes of both male and female subjects at a frequency of 25.2% and 74.8% respectively, the 1a/1a genotype was never observed in our female group contrary to our male group. The abovementioned *VN1R1* allelic differences potentially cause minor changes in the protein conformation and its transmembrane domains, as simulated by the TMHMM software. Given the equal distribution of both *VN1R1* 1a and 1b allelic frequencies in male and female individuals of our study sample and assuming that the functional properties of the two allelic forms of the VN1R1 protein are different, based on the protein conformation simulation, our data suggest that the absence of the 1a/1a genotype in females may correlate with distinct gender-specific behaviour.

P1161. Phylogeography of Y cromosome in northern and eastern Africa

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We have typed 893 male subjects from 23 populations in northern and eastern Africa with a set of 27 new and 74 previously described Y chromosome single nucleotide polymorphisms, and identified 40 different binary haplogroups. The analysis of molecular variance revealed a high and significant degree of Y-haplogroup interpopulation diversity (F_{st} = 0.23, P<0.0001). Upon grouping of the populations according to a geographic criterion, we obtained a F_{ct} = 0.12, (P<0.0001) and a F_{sc} = 0.16 (P<0.0001), indicating a high level of heterogeneity both among and within groups. The northeastern group of populations showed the highest degree of internal variation, a finding that could be only partially explained by genetic drift alone.

The majority of the populations analyzed speak languages belonging to 4 different branches of the afroasiatic linguistic family (berber, semitic, cushitic and omotic). When afroasiatic speaking populations were grouped following their linguistic affiliation, we observed a low and not significant level of apportionment of the variance between groups, suggesting that the afroasiatic linguistic branches spread independently with respect to genes.

Haplogroup E-DYS271, a haplogroup commonly found in sub-Saharan Africa, was found in most populations from northern Africa at frequencies around 5%. However, the analysis of 11-microsatellite-based network showed a very different haplotype distribution among the two regions, a finding consistent with the occurrence of relatively old trans-Saharan human movements.

Finally, the overall phylogeographic profile of E-M78 chromosomes revealed geographic partitions of sub-haplogroups that may indicate source and direction of human migrations between eastern and northern Africa.

P1162. Remarkable homogeneity of the human Y chromosome P lineage at the level of alphoid heteroduplex polymorphic system

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The Y chromosome alphoid heteroduplex (αh) polymorphic system is based on the simultaneous amplification of two-to-several loci (which differ by small point mutations and/or indels) in the centromeric alphoid region of the Y chromosome.This allows the generation of heteroduplex molecules (in addition to the normal homoduplexes) that can be seen as shifted bands in native poliacrylamide gels. The alphoid polymorphic system is highly polymorphic, and it can be generated by a combination of unique events (point mutations) and deletions/duplications in the right side of the alphoid block.

The αh polymorphic system was investigated in 135 Eurasian Y chromosomes belonging to six lineages (DE, G2, I, J, N, P*(xR1a), R1a). All the samples belonging to the P lineage presented the same αh2 haplotype and all the samples presenting αh2 haplotype belonged to P lineage. The same homogeneity can be observed in the clade G2, with all the samples belonging to αh4, but the small number of samples belonging to G2 clade prevents the inference of any conclusion. All the other analyzed clades (DE, I, J and N) showed heterogeneity at the level of the αh polymorphic system.

Despite the multi-mutational background of the αh system, we found no variation at all at this level for the P lineage, which confirms the known homogeneity of this lineage of the Y chromosome tree.

P1163. Phylogeography of Y-chromosomal lineages in Siberia and Cenral Asia

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The composition and frequencies of Y chromosome haplogroups, based on genotyping of 36 biallelic loci in its non-recombining part, was revealed in native populations of Siberia, Eastern Europe and Central Asia. 25 haplogroups were observed, but frequencies of only 7 of them were higher than 3 percent. In sum these 7 haplogroups comprise 86% of Y-chromosomal gene pool in population of North Eurasia.

The proportion of inter-population differences in the total genetic variability of region's population according to the analysis of molecular variance data is 19.04%. Inter-individual differences within populations account for the rest of total genetic diversity (81%). Analysis of genetic diversity within geographical groups reveals the high level of genetic differentiation in Eastern (Fst = 0.334) and Western (Fst = 0.300) Siberia. Male lineages in population North-East Asia and Central Asia are less differentiated. Slavic population of Eastern Europe in contrast to other regions are characterized by the uniformity of male gene pool (Fst = -0.0016).

Based on analysis of microsatellite haplotypes within main Y-chromosomal haplogroups, molecular diversity within monophyletic lineages were calculated and phylogenetic trees for most common haplogroups were reconstructed. Western-Eurasian lineages (R1a1, R1b) are characterized by the maximal diversity in Caucasoid populations. Among Siberian ethnic groups, the highest diversity of these lineages was found in Altay-Sayan populations, which probably reflects the presence of substantial amount of ancient Neolithic Caucasoid components in their gene pool. Eastern-Eurasian lineages have the high level of diversity of microsatellite haplotypes in populations of Eastern Siberia and North-East Asia.

P1164. Comparing Y chromosome haplotypes and surnames of Norse and Irish origin in men in Northern Ireland

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The paternally-inherited Y chromosome has been fundamental in understanding historic and pre-historic human migration patterns. Unlike autosomal markers, Y chromosome haplotypes are uni-parentally inherited and can therefore trace lineages. Y chromsome markers also have the advantage of being inherited in the same manner as surnames in some cultures, allowing for the comparison of cultural and genetic inheritance. Recent studies have shown that due to Ireland's relatively recent colonization by modern humans, coupled with its geographic isolation, it an ideal location for surname/Y-chromosome comparisons. Earlier work focused in the Republic of Ireland has shown the majority of Irish males (>98% in Connaught) belong to a single haplotype, R1b3. The remaining samples belong to haplogroup I. By contrast, a sample of the modern Norse population contains 30% Rlb3 and 28% I. This work suggests the possibility to genetically distinguish surnames of Irish-Gaelic and putative Viking (Norse) origin. We have begun typing 228 buccal swabs from men in Northern Ireland for 10 SNP (Single Nucleotide Polymorphisms) on the Y chromosome. Preliminary results show virtually no difference in men with Irish-Gaelic surnames: 63 of 68 samples (92.2%) in haplogroup PR* (inclusive of R1b3) and 5 of 68 in haplogroup GJ* (including I), and those with Norse surnames (83 of 90 in PR* or 92.6%, and 6 of 90 in GJ*). These results contrast with those seen in parts of Scotland and Northern England where the Norse and Danish Vikings had a more significant impact on the population.

P1165. The Bantu expansion: demographic features of the western and eastern waves of advance

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The present genetic patterning in Sub-Saharan Africa reflects the effects of one of the most important human pre-historical demographic movements. The dispersal of Bantu-speaking farmers from central-west Africa towards the south (along both the western and eastern coasts) is a typical example of a demic diffusion model of gene flow. The increasing availability of food allowed population growth, which in turn led to the need for migrating. Data collected until now suggest differences in the progression of the western and eastern waves of advance. Important questions remain concerning the numbers of people involved not only in the whole process, but also in the two main independent Bantu migration routes. In this study, we use likelihood analyses based on the coalescence theory (MIGRATE software) to evaluate the magnitude of the male migration rates and of the male population effective sizes involved in both waves of advance of the Bantu expansion. To investigate these, we analysed a collection of Y-chromosome biallelic and microsatellite markers in a population from South-western Africa and in a number of Sub-Saharan populations gathered from the literature that were in the path of both expansion waves. Estimated diversity indices, genetic distances and migration rates can be explained in light of the Bantu expansion. Together with phylogenetic analyses, our data support the hypothesis suggested by previous mtDNA analyses that the western stream of the Bantu expansion was a more gradual process than the eastern counterpart, which likely involved multiple short dispersals.

Po08. Genomics, technology, bioinformatics

P1166. Williams-Beuren-Syndrome: Determination of deletion-size using quantitative Real-Time-PCR

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The Williams-Beuren-Syndrome (WBS) a rare (1/20000-50000) genetic disorder, is usually associated with a 1.5-2Mb hemizygous deletion on chromosome 7q11.23. WBS-patients display vascular stenosis, weakness of connective-tissue, dysmorphic face, short stature and mental retardation. At least twenty-five genes have been identified in the deletion-region in WBS-patients, which is flanked by large low-copy-repeat sequences (> 320Kb).

Haploinsuffiency of the ELN-gene, LIMK1-gene and TFI-II-gene family has been shown to be causally involved in the pathogenesis of WBS. The flanking genes of ELN are assumed to cause additional features of WBS like mental retardation, hypercalcaemia and connective tissue abnormalities. By using FISH- and/or microsatellites analysis it is not possible to get a precise identification of the size of the deletions. For determining the deletion-sizes, we developed a reliable quantitative PCR-approach (qPCR). Our assay screens 2.5Mb of the WBS-region in 100-200 Kb intervals. This methodology has been tested in DNA samples of 65 patients with the clinical suspicion of WBS. In every case we were able to identify or to exclude the presence of a deletion and its size. Detected deletion sizes vary from 0.2Mb to 2.5Mb. This last rearrangement represents the largest described deletion and it was detected in a very severely affected patient. We report on the detection efficiency of this new system and on the genotype/phenotype-correlation.

P1167. Sequence Scanner Software v1.0: A Comprehensive, Effective and Free tool to View and Edit Sequence Traces

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With the availability of high throughput sequencing technology, researchers today are generating up to 250K samples per day. With such vast quantity of data the QC process, which involves identifying sample failures, tracking data anomalies and reviewing low quality samples can be time and labor intensive.

We present Sequence Scanner Software, a free tool (web download available at www.appliedbiosystems.com/sequencescanner) that allows researchers to navigate directly to failed samples and unique trends in data quality, with graphical result reports and multiple viewing options. For example, the thumbnails view is an effective way to quickly scan through large amounts of data and look for anomalies. To troubleshoot and inspect low quality bases within a trace a user can simultaneously view both the raw and the analyzed data peaks.

Sequence Scanner Software generates several trace quality reports such as trace read lengths in bar graphs making it easier and faster to evaluate data. In addition the reports contain hyperlink functionality that allow users to directly link between results and data. We demonstrate several features with Sequence Scanner Software that help provide an easy and effective data review workflow.

P1168. Regulation of 22q11 deletion syndrome genes during mouse development: expression microarray analysis

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22q11 deletion (DiGeorge/velocardiofacial) syndrome (22q11DS) is a developmental anomaly caused by an heterozygous interstitial chromosomal deletion. Although mouse models indicated Tbx1 as the gene responsible of the phenotype, the phenotypic spectrum of patients is complex suggesting that gene-gene and gene-environment interactions are crucial in delineating the pathogenesis of 22q11DS. In order to define cis-acting regulatory effects of 22q11Ds haploinsufficiency during development we designed a low density microarray (22q11DS-chip).

Expression level filtering and statistical analysis identified genes that were consistently differentially expressed during specific developmental stages (from 4.5 dpc to 14.5 dpc). Interestingly at each developmental stage all the genes that are above the threshold level (FC>±2) have a similar behaviour; in fact they are all or upregulated or downregulated (Tab 1). These experimental results have been complemented with a bioinformatic study of regulative sequence elements in the promoter regionof these genes.

Moreover eight genes are significantly expressed during all the developmental stages analysed; four are 22q11 genes (Pcqap, Ranbp1, Mrpl40, Top3b) and four are out of the 22q11 region (Pax3, Foxc2, Hoxa1, Hoxa3).

QRT-PCR validated microarray results.

The identification of 22q11 genes whose expression is dependent to specific development stages and that of 22q11 genes constantly expressed during embryogenesis, may be useful to understand the role of these genes in causing the 22q11DS clinical spectrum.This work was supported by a MIUR grant (Italian Ministry of University)

Tab 1

Developmental stage	Upregulated genes	Downregulated genes
4,5 dpc	-	Pax3; Hoxa1; Foxc2
6,5 dpc	Stk22b; Usp18; Txndr2	-
7,5 dpc	Usp18	-
8,5 dpc	Txndr2; Cldn5	-
9,5 dpc	Crabp1; Txndr2	-
11,5 dpc	-	Comt
14,5 dpc	Nlvcf; Ranpb1; Crabp1	-

P1169. High resolution mapping of DNA copy alterations in human chromosome 22 using high density tiling oligonucleotide arrays

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Deletions and amplifications of the human genomic sequence (Copy Number Polymorphisms) are the cause for numerous diseases and a potential cause of phenotypic variation in the normal population. Comparative Genomic Hybridization (CGH) has been useful as a tool for detecting large alterations in genomic DNA copy number. We have developed High-Resolution CGH (HR-CGH) to detect accurately the presence and extent of chromosomal aberrations in human DNA (1). Maskless array synthesis was used to construct arrays containing 385,000 oligonucleotides with isothermal probes of 45-85 bp in length; arrays tiling the b-globin locus and chromosome 22q were prepared. An array with a 9 bp tiling path was used to map a 622 bp heterozygous deletion in the b-globin locus. Arrays with an 85 bp tiling path were used to analyze DNA from patients with copy number changes in the pericentromeric region of chromosome 22q. Heterozygous deletions and duplications as well as partial triploidies and partial tetraploidies of portions of chromosome 22q were mapped with high resolution (typically up to 200 bp) in each patient, and the precise breakpoints of two deletions were confirmed by DNA sequencing. Deletions that had been undistinguishable by FISH were shown to be different in size. Additional peaks potentially corresponding to known and novel additional CNPs were also observed. Our results demonstrate that HR-CGH allows the detection of copy-number changes in the human genome at an unprecedented level of resolution.

1. Urban AE, Korbel JO et al. (2006) PNAS, in press

P1170. Phylogenetic analysis of the Apolipoprotein E family of proteins

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Among apolipoproteins, apolipoproteinE (ApoE) plays a pivotal role in lipid transport and is proposed to be involved in neural repair. Because of long divergence history of apolipoproteins, it is not known, however, how ApoE proteins evolved in time. Insight into their evolutionary relationships could profoundly improve our understanding of ApoE protein family. To investigate the evolution and relationships among ApoE proteins, we used the information from molecular data and analyzed phylogeny of ApoE proteins. The phylogeny of ApoE, as inferred from both the protein sequences and the corresponding gene sequences are compared. Appereantly a speciation event occurred that led to the formation of ApoE protein varients of human, monkey, olive baboon, chimpanzee, gibbon, and orangutan. ApoE of sheep, cattle, guinea pig, mouse and rat is placed separately in all trees built and thus are not very closely related to the ApoE of human and its sister taxa. ApoE sequences of fish and frog were found to be less related to the ApoE sequences of other taxa that were examined in this study. The most recent common ancestor of the ApoE is found to be the ApoE of frog.

P1171. Detection of Sub-Kilobase Sized DNA Copy Number Variants in the Human Genome Using Ultra-High Resolution Whole-Genome Array CGH

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Microarray-based comparative genomic hybridization (array CGH) methods have been used to investigate chromosomal abnormalities associated with cancer and developmental disorders on a genome-wide level. This method is also being applied for the investigation of copy number variants (CNVs) in the normal population. While several groups have reported on the presence of 10 Kb to several Mb sized copy number variants (CNVs) in the normal population, recent investigations indicate a high prevalence of CNVs down to ~0.5 Kb size

fragments. These studies suggest that CNVs play a significant role in phenotypic variation and are likely play a role in areas of human health such as drug metabolism and susceptibility to complex diseases. In addition to normal CNVs, it is anticipated that ultra-high resolution DNA copy number studies will reveal the presence of a significant number of disease-specific small-sized deletions and amplifications (e.g., in the case of running tumor and germline DNA from the same patient in cancer studies).
To investigate the full range of human genome variation, we have developed an oligonucleotide-based array CGH platform that contains 390K unique probes per array. A tiling-path array design format was used to systematically map copy number changes within genes and intergenic regions. Isothermal probes (target Tm = 76C), varying in length from 45 to 85 nucleotides, were used to enable detection of copy number changes in both AT- and GC-rich regions in the genome. Data will be presented on a set of cancer-free individuals at two levels of resolution (~1 and 20 Kb).

P1172. Development of a new array-MAPH methodology for detection of copy-number changes and screening of patients with X-linked mental retardation.

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Accurate and sensitive genome-wide screening to detect small genomic imbalances has been a technical challenge for a long time. The focus of this study was to introduce a novel methodology, applying the basic principle of Multiplex Amplifiable Probe Hybridization (MAPH) to a microarray-based approach for fast, accurate and reliable determination of copy-number changes of any loci in complex genomes. We have developed a new methodology and software for designing PCR-amplifiable hybridization probes (200-600bp) that can be used for both array-MAPH and array-CGH. We have designed, amplified, cloned and spotted onto arrays 560 target sequences for human chromosome X to cover it uniformly with median spacing of 238kb. Another 107 autosomal sequences were selected and used as normalization controls. For validation of the new methodology, several normal DNA samples and patient samples with known and unknown chromosome X aberrations were analyzed. Array-MAPH detected deletions and duplications, which were confirmed by PCR and/or FISH analyses, demonstrating the accuracy and sensitivity of the new approach. The new array-MAPH method was further applied for screening of 20 male patients from families with X-linked mental retardation (kindly provided by EURO-XLMR consortium). One deletion of approximately 500kb and two duplications of approximately 1.6Mb and 0.9Mb were detected and their segregation in the corresponding families was investigated. The new microarray methodology provides an alternative to array-CGH as well as several advantages for high-throughput diagnostic screening by enabling high flexibility to study virtually any region in the human genome.

P1173. Acute myeloid leukemia arrayCGH profiling reveals distinct categories within the genetic intermediate risk group

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Acute myeloid leukemia (AML) is an hematological tumor characterized by the clonal proliferation of undifferentiated myeloid precursors. Patients are classified into the favorable, intermediate or adverse risk group according to chromosome analysis. The intermediate risk group is characterized both by highly variable clinical outcome and genetic heterogeneity.
Objective: To characterize the genomic profile of the "intermediate cytogenetic risk group" AML at diagnosis by oligo-based arrayCGH,
Bone marrow DNAs from 88 cases of AML from the intermediate group were analyzed. All cases were previously clinical and chromosomally characterized. Molecular profiling was performed with a oligonucleotide-arrayCGH that contains 44K 60-mer probes and includes all known genes and positional probes at an average resolution 45Kb (manufactured by Agilent Technologies).
Results
1. ArrayCGH performed on DNAs from the AML series yielded a readable high resolution genome profile in all analyzed samples allowing the detection of additional changes in 17% of the samples.
2. 13% of the samples with normal karyotype showed single copy number changes: 4 deletions, 1 duplication. Chromosome regions affected by the changes were 2p23, 5q35, 6p24, 12q24, and Xp22, among others. Changes' size ranged between 2.8 Mb and 150 Kb
3. None of the 19 cases with numerical abnormalities (single and double trisomies) showed additional structural gains or losses.
4. 63% of cases with structural aberrations (as 20q-, 7q-9q-) showed an increased number of genomic aberrations after the arrayCGH analysis. The number of the aberrant chromosome regions was increased and their limits and nature of the changes have been characterized.

P1174. A genome-wide panel of non-synonymous SNPs for disease association studies

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Systematic studies of sequence variation in humans have allowed the compilation of a comprehensive list of circa 10 million common variants, predominantly SNPs. Exonic SNPs causing amino acid changes or STOP codon alterations (non-synonymous SNPs [nsSNPs]) are, by definition, very likely to have an impact on phenotype including disease.
We validated approximately 50,000 of the candidate nsSNPs available in dbSNP, using the Golden Gate assay (Illumina), in the HapMap CEU panel. Data were integrated with the publicly available Perlegen and HapMap validated SNPs and used to select a non-redundant set of 15,700 nsSNPs with frequency above 1%. The nsSNPs together with ~1300 tag SNPs from the MHC region were used to generate a custom chip for use with the Infinium assay (Illumina). The chip harbours 15,436 loci and can process six samples in parallel.
As part of the Wellcome Trust Case Control Consortium (WTCCC; http://www.wtccc.org.uk) we are using the nsSNP chip in an association study of four diseases (ankylosis spondylitis, breast cancer, multiple sclerosis and autoimmune thyroid disease) with 1000 cases per disease (national UK Caucasian samples) and 1500 common controls from the 1958 British Birth Cohort.
Analysis of 600 samples identified ~14,500 loci (95%) with good clustering and in Hardy-Weinberg equilibrium. Call rate for the above loci was greater than 99.8%. The controls are also being genotyped with the Affymetrix 500K chip arrays as part of a whole-genome scan WTCCC being conducted on eight other common diseases. Initial comparison of the 1500 SNP markers that are common to the two chips showed good concordance, ~99%.

P1175. Neuropeptide 'Semax' action on gene expression of BDNF and NGF in rat brain

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'Semax' is a short peptide, the physiologically active analogue of adrenocorticotropic hormone (4 - 10). It exerts neurotrophic and nootropic influence on the organism. This drug influences on the survival of neurons. It was shown previously that 'Semax' administration modifies neurotrophic factor genes expression. So the aim of our work was further investigation of 'Semax' action on the genome expression. According to the one of hypothesis 'Semax' stimulates the production of neurotrophic factors BDNF and NGF in neural tissue and increases the vital capacity of neurons. We observed it's influence on the expression of BDNF and NGF in rat retina and some parts of brain under the intranasal peptide introduction. The real-time PCR analysis showed that 'Semax' exerted different action on rat brain gene expression. BDNF expression increased reliably in rat brainstem (35%), cerebellum (57%) and hippocamp (46%). We also observed less valid increasing of BDNF expression in retina (46%) and decreasing of it in frontal cortex (24%). An analogous results were observed in NGF expression changes. The expression decreased reliably in rat frontal

cortex (52%) and increased in hippocamp (92%). There also was less valid increasing of NGF expression in brainstem (56%) and cerebellum (68%). So our investigation confirmed the neurotrophic 'Semax' action in vivo. Further investigation of it's influence on different genes expression could elucidate the exact mechanism of 'Semax' medicine action in treatment of hypoxia, cerebral ischemia and glaucoma.

P1176. The Gene Ontology Annotation (GOA) project at EBI

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The Gene Ontology (GO) is a well-established, structured vocabulary that has been successfully used for 8 years in the annotation of proteins. GO terms, created in consultation with the biology community, are used to replace the multiple nomenclatures used by scientific databases that can hamper data integration. Currently GO consists of more than 20,300 terms distributed over three ontologies that describe the molecular function, process and location of action of a protein in a generic cell.

The Gene Ontology Annotation (GOA) database (http://www.ebi.ac.uk/GOA) aims to provide high-quality manual and electronic GO annotations to proteins within the UniProt Knowledgebase. By annotating all 'known' proteins with GO terms and transferring this knowledge to highly similar 'unknown' proteins, GOA offers a valuable contribution to the understanding of all proteomes.

GOA provides annotated entries for almost 100,000 species and is the largest and most comprehensive open-source contributor of annotations to the GO Consortium annotation effort. In addition, by integrating GO annotations from model organism groups (FlyBase, GeneDB, HGNC, MGI, RGD, SGD, TAIR, Gramene, TIGR, ZFIN, AgBase, Reactome and IntAct), GOA ensures the dataset remains a key reference. GOA prioritises the annotation of the human proteome and fully supports the Human Proteomics Initiative (HPI), by focussing on the annotation of proteins involved in human health and disease.

The GOA dataset can be queried through a user-friendly web interface via our QuickGO browser (http://www.ebi.ac.uk/ego) or downloaded in a parsable format via the EBI (ftp://ftp.ebi.ac.uk/pub/databases/GO/goa) and GO FTP sites.

P1177. Detection of large deletions and duplications in genomic DNA using semi-quantitative multiplex PCR-based assay on capillary electrophoresis systems

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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 a semi-quantitative multiplex PCR-based method that uses relative quantitation of fluorescently-labeled short fragments. Fragments from BRCA1, BRCA2, 9p21 and MMR (MSH2) regions were amplified using FAM™-labeled primers 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. After normalization to a control amplicon, peak 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 system that can be used for both small and large-scale studies.

P1178. Construction and characterization of genomic libraries of *BRCA2* mutation carriers

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Mutations in the *BRCA* genes increase the risk of breast cancer. *BRCA1* and *BRCA2* are the two major genes responsible for the breast and ovarian cancers that cluster in families with a genetically determined predisposition. Recent studies showed that BRCA2 is involved in homologous recombination and DNA-repair.

Our previous studies have revealed multiple intrachromosomal rearrangements, duplications, inversions and deletions on 9p23-24 in lymphocytes of *BRCA2*$^{+/-}$ members from independently ascertained familial breast cancer clusters.

With the intention to define the localizations of the rearrangements and identify the involved DNA sequences we constructed three genomic bacterial artificial chromosome (BAC) libraries of three *BRCA2* mutation carriers. The lymphocytes derived from three different families with breast cancer history.

The libraries consist in total of about 100,000 clones with an average insert size of approximately 150kb. The insert sizes of the BAC-clones range from 60 to 280kb.

The libraries were screened for 9p-specific regions which showed chromosomal rearrangements in previous FISH experiments. The identified clones where end-sequenced, database-confirmed, size-analysed (pulse field electrophoresis) and used for FISH-mapping on metaphase-chromosomes from non-mutation carriers.

P1179. A Very Fast Run Module Optimized for Medical Sequencing for the Applied Biosystems 3730*xl* Series DNA Analyzers

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With the completion of the sequencing of the human genome, focus has moved to resequencing regions of medical research interest. Because the average human exon is less than 200 bases, the research strategy for medical sequencing has been to obtain short read lengths with high quality data. We have developed a very rapid run module and basecaller system for this application on the 3730*xl* DNA Analyzer. The new module uses the existing 3730 DNA Analyzer hardware and firmware, 36-cm capillary arrays, POP-7™ polymer, and the current BigDye® v3.1 sequencing kit-based chemistry. A new version of KB™ basecaller, version 1.3, has been developed for data analysis. This module is able to produce 400 to 500 bases of Q20 and greater data in less than 20 minutes run-to-run time. Nearly 7,000 samples can be run on one 3730*xl* Analyzer in one day, generating 2.8 million Q20 bases or more. The collection time can be further shortened for read lengths between 200 and 400 bases and these user editable modifications have been captured in a chart we have generated to help provide module modification guidance based upon read length needs. Sequencing data from the new module will be shown.

P1180. Functional study of transcription cis-regulatory elements predicted in the CDK5R1 3'UTR

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CDK5R1 encodes for p35, a neuron-specific activator of cyclin-dependent kinase 5 (CDK5), whose activity plays a central role in neuronal migration during CNS development and which has been implicated in several neurodegenerative disorders.

The remarkable size of CDK5R1 3'UTR prompted us to search for UTR regulatory elements which act on mRNA stability and translational efficiency, by means of the UTRScan bioinformatic tool. We predicted eight possible ARE (AU-Rich Elements), involved in transcript deadenylation/degradation, and a GY-box element, known to have a role in *Drosophila* post-transcriptional negative regulation of gene expression.

A Dual Luciferase assay was used to carry out the functional analysis: we cotransfected in SK-N-BE and HEK-293 cellular lines a *Firefly* luciferase expressing control plasmid and six overlapping fragments, covering the entire CDK5R1 3'UTR (C1-6), cloned in plasmids expressing *Renilla reniformis* luciferase at the 3' end of the reporter gene.

ARE containing C1 and C2 fragments showed a decreased luciferase activity in both cell lines, while ARE containing C5 and C6 fragments displayed similar levels of luciferase activity, compared to the control plasmid. The C3 fragment, covering the GY-box, showed high luciferase activity, suggesting for this element a function of transcript stabilizer in

human cells, differently from that evidenced in Drosophila. The ARE fragment C4, displayed reduced luciferase levels only in SK-N-BE cells, suggesting a line-specific post-translational regulation control.
The contribution of the predicted regulatory elements on post-transcriptional regulation mechanisms will be further elucidated by studying deleted/mutated fragments and performing degradation assays.

P1181. Simultaneous analysis and phase detection of the IVS8 Poly(TG) and Poly(T) Repeat Tracts in the *CFTR* Gene : comparison of three single step methods

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Precise genotyping at the IVS8 poly(TG) and poly(T) repeat tracts of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene may be of clinical relevance in the *CFTR* pathology. It has been demonstrated that length variations of both tracts influence splicing of exon 9, longer (TG)m associated with shorter (T)n repeats being less favourable for its efficiency. Identification of (TG)12 or (TG)13 repeats adjacent to (T)5 could be essential, as these haplotypes are more likely to be associated with an abnormal phenotype. As a result of growing interest, several assessment methods have been developed, most of them multistep, time consuming and not always designed to determine the phase.
We here present a comparison of three molecular methods of simultaneous poly(TG) and poly(T) genotyping which rely on a single step assay and allow direct phase detection, thus avoiding family linkage study. Genomic DNAs from 75 patients referred to our laboratory and previously studied with our routine method based on denaturing gradient gel electrophoresis (DGGE), were analysed by a following protocol recently described based on melting curve analysis of hybridization probes combined with real-time PCR. We then applied a third method newly developed which relies on a fluorescent multiplex alleles specific PCR : the 5, 7 and 9 poly(T) repeats are specifically amplified and detected after fragment analysis on an ABI sequence analyzer. Exact poly(T) and poly(TG) lengths are determined by colors and sizes of PCR products respectively. Advantages and disadvantages of each method are discussed

P1182. Genomic divergence between mouse and man visible from Vega

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Approximately 80% of mouse genes have a single known orthologue in human in large blocks of sequence, however there are several regions where this is not the case. A region of extensive micro-rearrangements is present on mouse chromosome 11 (mm11) orthologous to part of human 17 (hs17) where the region is involved in two neuropathies (Charcot-Marie-Tooth Disease and Smith Magenis Syndrome). Conversely, a region on mm4 which is associated with various deletions (the so-called brown deletions) is spread over different parts of the equivalent hs9 and also displays, in mouse, a micro-duplication and unique tandemly duplicated genes. Further we observe a number of gene clusters such as keratin, keratin associated protein, major urinary protein, prolactin and vomeronasal receptor, that exhibit considerable difference in gene number between mouse and man (usually expanded in mouse). These have specific functions and are involved in mating/reproduction or the sensory mechanism.
Comparison of these regions between species requires accurate manual annotation on finished sequence. Because of the nature of these genes and regions automatic annotation, such as generated by Genewise (used by Ensembl) or Pairagon, generally does not suffice, neither on the structure nor on the nomenclature level. For example pseudogenes, important when comparing clusters, are mostly absent. The Havana group at the Sanger Institute performs manual annotation on human, mouse and other chromosomes. The results are accessible through the Vega web browser, an Ensembl derived genome annotation viewer. Vega can display the annotation of equivalent human and mouse regions simultaneously through the MultiContigView interface.

P1183. An optimized aCGH protocol permits reduced genomic DNA input

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The use of microarray technology has been growing rapidly over the past decade. Array comparative genome hybridization (aCGH) has become the method of choice for the detection of copy number changes in tumors and genetic disorders. When working with patient samples, the amount of target material available for use on arrays is often limited. We have developed a new protocol for preparing genomic DNA samples to be hybridized to our 60mer oligonucleotide CGH microarrays. The improved method does not require a DNA amplification step and the digestion and labeling reactions have been streamlined resulting in a shorter processing time without compromising array performance. Using this optimized workflow we can reliably hybridize as little as 500 nanograms of genomic DNA to arrays while maintaining high quality results. The protocol has been validated on our aCGH microarray platform in experiments using DNA from commercial sources, DNA isolated from cell lines, and DNA isolated from formalin-fixed, paraffin-embedded tissues. Normal male/female DNA comparisons yielded ROC areas (a measure of true positives versus false positives) of greater than 0.98, indicating a high degree of accuracy. Analysis of DNA isolated from colon carcinoma cell line HT29 (ATCC) successfully detected previously identified aberrations in chromosomes 8 and 16. Preliminary results from cell line and FFPE samples indicate the protocol may be useful with even lower input levels.

P1184. Two novel methods for rapid quantification of human complement C4A and C4B genes

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The fourth component of human complement (C4), an essential factor of the innate immunity, is represented as two isoforms (C4A and C4B) in the genome. The C4A and C4B genes, encoding the two isoforms of complement 4, are located in the chromosome 6, and manifested by variable copy numbers among individuals between zero to six in the human diploid genome. Quantification of the C4A and C4B genes has great clinical importance since unbalanced production of C4A and C4B is associated with several diseases. High throughput analysis methods for C4 gene dosage determination are not yet available.
Here, we show two new, rapid high throughput genotyping methods for the determination of the number of the complement C4A and C4B genes. The first method based on real time PCR, and affiliates the two major applications of TaqMan probes: quantitative assay and SNP detection (Szilagyi, 2006). The second method a novel combination of allele specific PCR and capillary gel electrophoresis (CGE) separation for rapid quantification of the C4A and C4B.
These methods enables automated and high throughput gene dosage analysis, especially in large scale populations screening.

P1185. Copy number detection by gene dosage assays using TaqMan® real-time PCR

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Gene copy number variation is becoming recognized as an important type of polymorphism in human genome. It is believed that differences in gene copy number could be a significant source of genetic variation between individuals. Gene copy number polymorphisms have been associated with genetic diseases such as cancer, immunological and neurological disorders. Gene duplication or deletion can have a significant impact on phenotype. For example, copy number changes for drug metabolism genes such as GSTM1, GSTT1, and CYP2D6 are known to be associated with variations in phenotype. Developing robust and accurate assays to detect copy number change will help to understand the role of gene dosage variation in human genetic disease and alterations in metabolism. Here we report the development of real-time quantitative PCR assays to quantify copy number using TaqMan® technology. The method involves relative quantification of the gene of interest versus a reference gene known to be single copy. Relative quantity is determined by the ΔΔCt method, where the endogenous

control is RNase P, and the calibrator is a DNA sample used as the basis for comparative results. Gene copy number is 2 x relative quantity. We have developed assays to measure gene dosage in a variety of genes, including 4 important drug metabolism genes (CYP2D6, CYP2E1, GSTM1 and GSTT1). The TaqMan-based duplex gene dosage assay is more reproducible, accurate, and robust for copy number detection compared to other methods.

P1186. Universal Detector assay for measuring DNA copy number changes

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Understanding the genetic basis of human phenotypic differences requires the study of an increasing variety of human genetic variations. Detection of single nucleotide polymorphisms (SNPs) has been at the center of efforts to characterize the genetic components of diseases and traits. However, emerging evidence suggests that intermediate and large-scale DNA copy number changes in a genome are prevalent and account for an important source of genetic variation between individuals. Several methods have been developed to measure DNA copy number changes. Most of them require the enzymatic manipulation of genomic DNA and the analysis of fluorescently labeled DNA fragments by either array hybridization or capillary electrophoresis. We report here a new assay called Universal Detector (UD) for accurately measuring copy number changes. The UD assay utilizes a 96-plex oligonucleotide ligation assay (OLA) followed by 48 duplex TaqMan(PCR reactions for analyzing up to 96 genetic loci. Our results show an accurate identification of duplicated sequences in samples with known chromosome duplications. The UD assay further has a linear performance if a sequence is amplified up to 8-fold, whereas higher fold changes are slightly overestimated. In addition, the UD assay is quantitative for less than 2-fold copy number changes. It can be completed within a day and is suitable for genetic studies requiring a low to medium sample throughput. UD is a powerful, new tool for accurately measuring DNA copy number changes.

P1187. In silico search for cryptic RSS with high recombination potential in the human genome

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When the system of V(D)J-recombination gets out of control the protein complex RAG1/2 can break of DNA outside human Ig and TCR genes. The resulting deletions and translocations damage some genes (HPRT, SCL, etc.). In this case sites targets of enzymes are cryptic recombination signals (cRSS) which are not structurally identical to recombination signal sequences (RSS) of Ig and TCR genes. At present no information is available about the quantity and intragenic location of cRSS whose structure has a high recombination potential and the number of theoretically possible intragenic deletions and inversions with the participation of such cRSS.

Having researched the annotated DNA sequences of 24 chromosomes in silico we have discovered 5.6 mln of 12 bp and 23 bp spacer cRSS outside the Ig and TCR loci. Their number is 1.5 times bigger than the theoretically expected value. On average the discovered structures make about 6 % of genome DNA. Having analyzed the nucleotide composition of heptamers and nonamers we have discovered that 5696 cRSS have a high recombination potential. 88 and 2383 of them are located in exons and introns of 2207 genes that code proteins and RNA. With the use of 12/23-bp spacer rule we have found out that such cRSS can theoretically participate in the formation of deletions of exons in 70 genes and in the formation of inversions in 86 genes. 23 genes can suffer from both type of damage. We are planning further to check the existence of the assumed gene damage in vivo.

P1188. Introduction of Conformation Sensitive Capillary Electrophoresis as a reliable tool for mutation detection in routine DNA-diagnostics

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Although large-scale mutation analysis by sequencing is now possible and very accurate, it still is relatively laborious and expensive. Current indirect scanning methods such as DGGE and dHPLC are reliable, but the throughput is limited. We have studied whether Conformation-Sensitive Capillary Electrophoresis (CSCE) using the new POP™ Conformational Analysis Polymer (CAP, Applied Biosystems) might be a fast and reliable alternative. Using this polymer under semi-denaturing conditions on an automated sequencer (Applied Biosystems 3730) we validated 243 different mutations in 14 genes, including 74 mutations in *BRCA1*, 78 mutations in *BRCA2*, 43 mutations in *CHD7* (CHARGE syndrome) and 9 mutations in the mitochondrial DNA. All mutations were correctly identified due to a shift in the peak pattern. For mitochondrial DNA, mutations present at 10% heteroplasmy could easily be identified. Several parameters were tested to further optimise the method, including the length of the fragment and the use of common primers for labelling of the fragments. As CSCE allows the possibility of multiplexing, at least 4 fragments can be simultaneously analysed in each capillary in one sequencer run (2 hours, 50 cm capillary). For a 48 capillary sequencer this results in a throughput of approximately 2300 fragments within a twenty-four hours' period. Data-analysis is also straightforward since the introduction of dedicated BioNumerics software. We conclude that CSCE proves to be a fast and reliable method for routine mutation detection in a diagnostic setting. Furthermore, as no optimization is needed, this technique can also be used for the rapid analysis of candidate genes.

P1189. The Mouse Genome Informatics (MGI) Database: using the mouse to decipher the genetic etiology of human disease

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The MGI Database (http://www.informatics.jax.org) represents an integrated, in-depth resource of genetic, genomic and biological data for the laboratory mouse. MGI maintains a pivotal role in comparative phenotype analysis through full integration of rich biological data sets, ultimately aiming to grant effortless navigation through sequence, expression, mapping, biochemical process, phenotypic outcome and disease model information. Recent enhancements capitalize on the robust annotation of aberrant mouse phenotypes in the context of mutations, strain variations, QTLs, and complex traits that serve as putative models of human genetic diseases, with supplementary phenotype-related images, where possible. Enhanced querying parameters include phenotype search terms from controlled vocabularies that circumvent the limitations of data retrieval through textual searches. Hence, the Mammalian Phenotype (MP) Ontology continues to evolve as a standardized, structured vocabulary that permits phenotypic representation across different domains and species, and supports annotations to individual genotypes (allelic combination(s) plus genetic background) at varying degrees of granularity. Application of hierarchical MP terms fosters new routes to map molecular functional features of gene products to complex phenotypic descriptions, and facilitates semantic interpretation and data mining from either a genotype or phenotype standpoint. Likewise, use of the Human Disease Vocabulary Browser, a dynamic set of disease terms from OMIM (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), optimizes access to human disease data for which Mendelian inheritance is suspected or proven. Links to OMIM entries are used to harness associations between observed mouse phenotypes and orthologous human gene mutations or disease syndromes for which distinct mouse genotypes phenomimic the human condition. Supported by NIH/NHGRI grant HG00330.

P1190. Development of a screening tool to validate gene sets obtained from global screens

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During the last decade, different approaches had been developed to identify prognostic or diagnostic marker genes. Especially microarray based expression analysis led to the definition of gene sets, the expression patterns of which were predictive for certain disease phenotypes. A synergy between these advances and the development of screening tools, which allow a rapid, reliable, and reasonably priced screening of marker gene expression represents an important step towards an improved treatment strategy. For a rapid and reliable semi-quantitative expression analysis of eleven candidate genes for drug resistance in melanoma, we combined a multiplex RT-PCR (mRT-PCR) approach with subsequent microfluidic fragment analysis. The functionality of this approach was demonstrated by low inter-experimental variations of amplicon quantities after endpoint analysis. Applied to RNA samples derived from drug-sensitive and -resistant melanoma cell lines, the multiplex RT-PCR delivered results qualitatively concordant with data obtained from Northern blot- and array-analyses. Further tests using an automated on-chip electrophoresis platform indicate the applicability of this approach for high throughput measurements. In conclusion, we developed a rapid and reliable screening tool to validate gene sets obtained from global screens.

P1191. Deletion/Duplication screening of the *DMD* gene in 98 individuals using MLPA technique

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MLPA, multiplex ligation-dependent probe amplification, is now widely recognized as a reliable method to detect both deletions and duplications in several genes, including *DMD* [MIM 300377]. Mutations in this gene are responsible for one of the most common neuromuscular disorders - the allelic Duchenne and Becker Muscular Dystrophies (D/BMD) [MIM 310200 and 300376].

In our laboratory, the commonly used multiplex PCR reactions, Southern blot and hybridization techniques have been used in the routine molecular diagnosis of D/BMD. In total, there were 373 unrelated referrals, including cases later seen to have been misdiagnosed, presenting defects in other genes (ex: sarcoglycans, *CAPN3*). These methods enabled the characterization of *DMD* deletions in 137 patients of which 45% involved exons 45-52, 13% exons 3-19 and 16% a single exon (either 44 or 45).

We used MLPA assay to screen for mutations in 68 unrelated patients (partially retrospective study). All 21 deletions were confirmed, and a further 15 duplications were detected (8 undocumented changes), two of which were already suspected based on routine screening results. Additionally, carrier status was ascertained and/or clarified in some families, also enabling determination of *de novo versus* familial mutations in sporadic cases, as well as the detection of gonadal mosaicism.

The authors highlight the added value of complementing routine methods with the MLPA technique in the molecular diagnosis of D/BMD, considering that gross deletions and duplications comprise the majority of mutations in the *DMD* gene.

P1192. Improving the accuracy of mutation detection by DNA sequencing using novel approaches to base calling and data representation

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A critical factor for the detection of mutations or heterozygous sequence by automated DNA sequencing is the accuracy of automated base calling. Automated base calling accuracy is compromised by the variable incorporation rates of di-deoxynucleotides and subsequent variation in sequence peak heights. In some cases one of the peaks at heterozygous positions may be so low it cannot be discriminated from background and maybe miscalled. Importantly this small peak is usually approximately half the height of a homozygous peak of the same base at the same position. We have developed a base calling algorithm that is a feature of our Assign-ATF software that improves the accuracy of automated base calling and also enables semi quantitative applications of automated DNA sequencing. Our system exploits the reproducible nature of re-sequencing. If the same position is sequenced in different individuals the relative peak heights of the same bases at the same positions are almost identical. Our approach normalizes the sequence data and represents the peaks relative to what is expected for a homozygous peak at each position. This results in homozygous peaks being the same height as each other and heterozygous peaks being 50% of the homozygous peaks. Our software also dynamically calculates and subtracts the background. Together these algorithms dramatically improve heterozygous base calling and minimize base call errors. We have used this approach to sequence DNA pools to simultaneously screen for and compare the frequency of SNPs between different populations and to detect low level mutants in HIV drug resistance genotyping.

P1193. Living cell irradiation and double-strand breaks

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Keywords: microprobe, protons, double-strand breaks, fibroblasts.

Single-particle microbeam facilities with their abilities to irradiate a single, well localized cell (or even cell compartments) with a defined number of particles are an excellent tool in radiation biology research. They give the possibility to analyze the end points as well as the underlying mechanisms. The aim of the study was to analyze the cell damage induced by proton irradiation.

Human skin fibroblasts were seeded into specially designed Petri dishes, with a 3 x 3 mm^2 Si_3N_4 irradiation window (to insure the protons passage), 1 day before the experiment. Cells were irradiated at Krakow microprobe facility with 2 MeV protons from the Van de Graaff accelerator with three different doses: 1 Gy, 6 Gy and 10 Gy. Following a standard immunohistochemistry procedure, cells were blocked with H2A.X monoclonal primary antibody and alexa fluor 488 secondary antibody at a concentration 1:500 for 1h at r.t. Double strand breaks (DSBs) foci were scored under the fluorescent microscope. The results showed a 4.2 fold increase in DSBs formation at 1 Gy, 8.3 fold at 6 Gy, and 11.5 fold at 10 Gy samples in comparison to the control. The DSBs formation correlated positively with the dose increase. The obtained results strongly suggest further continuation of these studies.

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P1194. GeneExpress: a design study for a pan-European research infrastructure dedicated to gene expression mapping of early human development

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Characterising gene expression patterns is a crucial part of understanding the molecular determinants of development and the role of genes in disease. However, this exciting area suffers from fragmentation of efforts across Europe, from difficulty of sourcing and maintaining suitable collections of material, and in developing expertise in both biological and informatics areas. Moreover, due to the special nature of the material involved in the field of human development, it is essential that ethical aspects are carefully considered.

In April 2005, the University of Newcastle was granted the coordination of GeneExpress, an EU-funded project in framework programme 6.

The Euro2.2m Design Study aims to evaluate the most effective ways of overcoming challenges faced by the community of scientists involved in the analysis of gene expression in early human development.

GeneExpress will build on European strengths in grid technology and developmental gene expression studies. Currently, there is no international infrastructure to bring these two very different fields of expertise together.

GeneExpress multidisciplinary team will define the organisational and collaborative structures, the ethical framework and the molecular and genetic technologies and informatics technologies necessary for a new research infrastructure.

P1195. EGFR mutation to quinazolin inhibitors response using cheminformatics tools

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Mutations of the EGFR gene have been identified in specimens from patients with non-small lung cancer who have a response to quinazolin inhibitors such as CI1033, Geftinib, and Erlotinib. Substitution mutation G719S was reported, this mutation is located in the GXGXXG motif of the nucleotide triphosphate binding domain (P-loop). This mutation mediates oncogenic effects by altering downstream signaling and antiapoptotic mechanisms. This glycine residue is mutated to Serine residue by using in silico technique. The 3-D structure of the mutant EGFR (G719S) was built by using crystal structure coordinates of SYK (1XBC.PDB). Then the model structure was further refined by energy minimization and molecular dynamics method. The active site of the mutant EGFR structure was analysed and compared to EGFR crystal structure (1M17.pdb) with respect to structural difference. The surface area of mutant EGFR and WT EGFR active sites are 657.73 A^0.2/U.C for 466.36 A^0.2/U.C. respectively. The interaction energy of the quinazolin inhibitors with each individual amino acid in the active of EGFR is calculated by the advanced program Affinity. The overall binding affinity of the ligand molecule was slightly affected, but surprisingly the mutated serine residue is found have better interaction with ligand molecule except Erlotinib. The measured values are improved for electrostatic and vanderwaal's interactions. The values are given in the table.

	WT EGFR Activity (GLY_695)			Mutated EGFR Activity (G695S)		
	CI1033	Geftinib	Erlotinib	CI1033	Geftinib	Erlotinib
Electrostatic (E ele)	0.184*	0.066	-0.139	-0.193	0	0.176
Vanderwaal's (E vdw)	-1.061	-0.23	-0.812	-1.311	-1.31	-0.962
Total (E Total)	-0.877	-0.161	-0.951	-1.504	-1.314	-0.786

*---values are measured in Kcal mol $^{-}$

P1196. A bioinformatic tool for the medical research

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GENATLAS is a database which provides information including the structure, expression and function of human genes, their mutations and the corresponding genetic diseases. Recently, a new therapeutic approach for the Duchenne muscular dystrophy gave good results on the mouse. It is based on the exon skipping technique and consists in removing at the mRNA level the exon which is responsible for the disease, allowing the production of a shorter but however functional protein. We developed at GENATLAS, in partnership with the AFM, an innovating data-processing tool to search for other candidate genes for this gene therapy. It makes it possible to carry out a research on the whole genome by associating different criteria of selection for the exon skipping technique, like the length of the mRNA transcript, the number of exons, the localization, the function of the protein in the organism. For each gene matching, a short description and a visualization of the organization of its exons are immediately given. By a code color, it is then possible to determine which exon can be skipped while the transcript remains within the correct reading frame in order to produce a functional truncated protein. A link on the protein reference source gives access to its complete description on NCBI site. Each record of a gene is linked to the GENATLAS database and to its associated phenotypes. The researchers thus have a fast tool for pre-selection of the candidate genes for the exon skipping technique in order to apply this new therapeutic approach.

P1197. Towards an SNP-based human profiling across population studies

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Single nucleotide polymorphisms (SNPs) are likely in the near future to have a fundamental role in forensic fields and human population studies. Forensic DNA analysis is routinely performed using polymorphic short tandem repeat (STR) markers, however SNPs could be more successful for degraded/minute DNA samples. Basically, a number of four SNPs is required for each STR polymorphism to reach the actual P^d (power of discrimination).

We have selected a panel of 50 SNPs aimed to the development of a SNP-based human identification system suitable for forensic purposes. The SNPs have been selected from the dbSNP (http://www.ncbi.nlm.nih.gov/). Selection criteria were mainly focused on allelic balance and specificity. Frequencies of the selected markers are being estimated on a sample of 700 European (Italian) individuals, 200 Africans and 200 Asiatic, the most of them already typed by traditional STR-based profiling kit, and compared to the frequencies reported by the HapMap project (http://www.hapmap.org/). All the samples are being typed using the Real Time PCR technology (Applied Biosystems), and results confirmed by direct sequencing. Interestingly, some of the frequencies in the Italian population showed discrepancies from those reported by HapMap as demonstrated by the SNP rs675236 which showed a highly significant difference in allelic and genotypic frequencies (p-value of 0.0007). These data reveal that frequencies available by HapMap project could show significant differences across the populations considered as the same ethnical group suggesting that generation of population specific frequencies is mandatory for medical genetics and forensic studies.

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P1198. TaqMan® Low Density Array: Gene Expression Analysis using Human Immune Profiling and Human Endogenous Control Gene Signature Panels

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The TaqMan® Low Density Array allows for rapid screening of many samples with 10s to100s of TaqMan® Gene Expression Assays. The researcher can move from gene lists and single assays in tubes to large scale real-time PCR gene expression profiling without the the need for liquid handling robots. Of particular concern to researchers is assay performance on the Low Density Array relative to plates, as well as the precision within arrays, across arrays and between manufactured lots. To compare performance between plate and Low Density Array, we looked at data generated from > 3000 TaqMan® Assays that were run in parallel on TaqMan® Low Density Arrays and 384-well plates. These data show that assays on the array can discriminate 2-fold change with similar sensitivity as on plates. We used data from multiple manufactured lots of the Human Immune Profiling Gene Signature Panel to determine assay reproducibility and found that across arrays reproducibility was very good; across manufacturing lots the standard deviation increased but was well within the QC standards set for within array reproducibility. In these studies we show the performance parameters of TaqMan® Arrays and the results of 32 tissues run on the TaqMan® Low Density Human Endogenous Control Array Gene Signature Panel.

P1199. Copy number determination using the SNPlex™ genotyping system

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Genome copy number changes (CNPs) are far more frequent than originally expected, and many of them affect gene copy numbers. Several genetic disorders are the result of CNPs, however because of technical limitations, the extent to which such contribute to phenotypic variations is still poorly understood. We recently introduced the SNPlex™ Genotyping System to address the need for accurate genotyping data, high sample throughput, study design flexibility, and cost efficiency. The system uses oligonucleotide ligation/polymerase chain reaction (OLA/PCR) and capillary electrophoresis (CE) to analyze single nucleotide polymorphism (SNP) genotypes (Tobler et al. J. Biomol. Tech. 16(4), 2005). Here we demonstrate the feasibility of an adaptation of the SNPlex Genotyping System, to analyze CNPs by comparing the intensity ratios of OLA reactions in test and reference regions. We will present the copy number analysis of DNAs with known chromosomal duplications. Specifically, we studied 88 genomic DNAs, 7 of which contained duplications of the chromosomes 9, 13, 18 or X. On each duplicated chromosome we analyzed at least 10 test OLA reactions, and differences in intensity ratios confirmed all known chromosomal duplications. The assay further identified male (XY) and female (XX) DNA samples due to their copy number difference of the X chromosome.
In addition to identifying known chromosomal duplications, we analyzed copy numbers changes of the RCCX module of the human MHC complement gene cluster.

P1200. Identification and functional characterization of novel factors regulating cellular cholesterol metabolism

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Hypercholesterolemia is an important risk factor for atherosclerosis and coronary heart disease. Current pathophysiological models of hypercholesterolemia assume a tight association of environmental as well as genetic factors, many of which a yet unknown. For the identification of genes predisposing for hypercholesterolemia, we here present progress on the establishment of a combined expression profiling and microscope-based functional screening approache that will allow us to systematically identify new candidate genes which are regulated by cholesterol and fatty acids and themselves are involved in regulating cellular lipid metabolism. Central feature of our approach is the use of microscopic cDNA- and RNAi-based functional cell arrays, a high-content screening microscopy platform and automated image analysis software. Altogether, this technology allows gain-of-function and loss-of-function studies in cultured cells with a high throughput and up to a genome-wide scale. Our study aims towards a more comprehensive understanding of the molecular basis of cellular sterol regulation, with our methodology being suitable for addressing a wide range of biological and medical questions.

P1201. Peripheral expression variability of FBN1 and TGFBR2 genes in genotyped patients with Marfan Syndrome.

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FBN1 (MIN *134797) and TGFBR2 (MIN +190182) mutations can cause Marfan syndrome (MFS). One of the unexplained features of MFS is the pathogenic mechanism that leads to marked inter- and intra-familial clinical variability, and also a variable disease penetrance. We tested the hypothesis that FBN1 and TGFBR2 genes are differentially expressed in patients diagnosed with MFS carriers of FBN1 or TGFBR2 gene mutations respectively, and that the differential expression can be measured also at the peripheral level.
Total RNA was extracted and amplified with two sets of TaqMan probes respectively for the 5' and 3' of the each gene.
RNA analyses identified:
1. In the paediatric patients (<18 years old) either with FBN1 or TGFBR2 mutations, the expression values of both genes are from 10 up to 60 times fold higher respect to the adults (affected or unaffected) and 5 to 20 times fold higher respect to unaffected young.
2. In the FBN1 patients harbouring PTC mutations in the 3' of the gene (from exon #50 to exon #65) the mRNA levels are as in unaffected patients.
3. In FBN1 patients harbouring mis-sense mutations the expression levels is pretty variable, according to the mutation location.
4. In TGFBR2 patients with mis-sense mutation, the mRNA levels are 4 to 8 times higher with respect to unaffected controls
We suggest that differences in normal FBN1and TGFBR2 expression could contribute to elucidate the mechanisms of the clinical variability seen in thees families with MFS.

P1202. Methylation-sensitive microarray CGH detection system for genome-wide analysis of DNA methylation

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Difference in DNA methylation is an essential element in several epigenetic regulations. Allele-specific gene expression dependent on parental origin that is called genomic imprinting is associated with differentially methylated regions (DMRs) - chromosome loci, where methylation status differs between paternal and maternal alleles. In the present research, we applied genome-wide, BAC-based microarray for the analysis of DNA methylation in different tissues and for the determination of novel DMRs in human. We designed a methylation-sensitive detection system, and, to estimate its efficiency, developed custom microarray covering imprinting control centre at the Prader-Willi syndrome/Angelman syndrome region at 15q11-q13. The system successfully identified BAC clones corresponding to the reported DMRs. Then, we screened the human genome for novel DMRs using genome-wide microarray (2178 clones spaced 1.4 Mb in average) and DNA from tissues of complete hydatidiform mole (androgenesis) and benign ovarian teratoma (parthenogenesis). Both tissues are uniparental diploidies; therefore complete hydatidiform mole and benign ovarian teratoma are presumably keeping only paternal and only maternal primary imprints, respectively. DNA extracted from placenta, lymphocytes and sperm served as controls. As a result of hybridisation experiments, we obtained general methylation patterns for different tissues. We have also confirmed the results of microarray analysis in several clones that cover regions with different methylation status in experimental tissue samples. Methylation-sensitive microarray CGH system detects difference in DNA methylation among tissues; this could help to identify novel DMRs in human genome.

P1203. MicroRNA expression analysis in Trisomy 21 and normal individuals

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Down Syndrome or Trisomy21 is a disorder due to an extra copy of chromosome 21. Our understanding of the molecular pathogenesis 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 have used quantitative real-time PCR to accurately measure the expression of miRNAs. Due to their small size (~22nt) and the absence of poly(A) tail, a specific assay was designed to reverse transcribe

each mature miRNA with specific stem loop primers. We analysed the expression of 3 HSA21 miRNAs (miR-125b, miR-let7c, miR-155) and 5 miRNA non-HSA21 in lymphoblastoid cell lines from 14 normal and 14 trisomic unrelated individuals.
Our results show a significant variability in miRNA expression among normal and trisomic individuals, suggesting a potential regulatory influence of the genetic background. The average values obtained for the normal versus the trisomic lymphoblastoid population were not different for the non-HSA21 miRNAs. Surprisingly, no significant overexpression was found for the HSA21 miRNA in the lymphoblastoid cell lines of trisomic individuals. Thus, we conclude that in these cell lines the miRNAs may not contribute to any cellular phenotypic difference between the 2 groups. We are currently analysing the expression of these miRNAs in different tissues in order to determine if their expression is controlled by additional tissue-specific regulatory mechanisms.

P1204. Identification of microRNA and mRNA marker genes for monitoring ES identity and differentiation in mouse

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A total of 248 microRNAs (miRNAs) were quantified using TaqMan® miRNA assays in 13 mouse embryonic stem (ES) cell lines, 30 differentiated embryoid bodies (EBs) and 6 mouse tissues. MicroRNA expression profiles can classify ESs, differentiated EBs and adult tissues. We have identified ES- and differentiation-specific miRNAs that could be used as biomarkers to determine ES cell identity and to monitor the differentiation. There exists a highly conserved miRNA expression signature in 13 ES lines. Only ¼ miRNA genes are highly expressed in ES cells, and during development an increasingly elaborate miRNA signature is expressed. The stem cell specific expression of a small set of miRNAs is lost in an apparently coordinate fashion during development and does not reappear in any somatic lineage. Based on the elucidation of this regulated miRNA molecular signature, it seems likely that there is a significant role for miRNA action in the early embryo. Of 33,381mRNAs probed in an AB 1700 microarray, 452 are differentially expressed between ES cell lines and EBs. Identification of these ES differentiation-related miRNA and mRNA marker genes could be used to examine ES cell identity and monitor the spontaneous differentiation of ES lines during cell culture.

P1205. Quantitative microsphere hybridization with single copy probes: More accurate than genomic and expression microarrays

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Genomic copy number can be determined directly by quantitative microsphere suspension hybridization (QMH). Computationally-defined single copy (sc) genomic fragments were conjugated to spectrally-distinct polystyrene microspheres and used to quantify homologous sequences in labeled genomic DNA by flow cytometry. Copy number differences were determined by comparing the mean fluorescence intensities (MFI) of test probes with a diploid sc reference probe in patient samples and abnormal cell lines. One, two and three alleles were readily distinguishable regardless of chromosomal context: (a) the MFI ratios of *ABL1* probes were reduced by 0.59 fold in patients with del(9)(q34q34) and increased 1.42 fold in trisomic 9 cell lines; (b) *TEKT3* and *PMP22* probes detected proportionate copy number increases in CMT1a patients with dup(17)(p12p12); (c) a chromosome 15q11.2q13 probe array identified imprinting center and common deletions in Prader Willi and Angelman syndrome patients.
Additionally, QMH with C_ot-1 DNA was used to compare genomic hybridization to sc probes in the presence or absence of adjacent repetitive elements. Contrary to expectation, C_ot-1 enhanced hybridization by 2.2-3 fold to sc probes with adjacent repetitive elements, because C_ot-1 is enriched for linked sc sequences that distort quantification of hybridization to genomic targets. We found that genomic and expression hybridization microarray measurements using C_ot-1 with repeat-containing probes were less reproducible than for sc probes. Systematic error in the design of genomic hybridization experiments was mitigated either by use of sc probes alone or by substitution of synthetic repetitive elements present in probe sequences for C_ot-1.

P1206. Increasing the mutation detection rate in DNA diagnostics by rapid implementation of MLPA using synthetic probes

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Sequence analysis will stay the golden standard for the identification of genomic mutations during the forthcoming years. However, this technique has a major drawback, since heterozygous (multiple) exon deletions and duplications will not be detected. Recently, deletion/duplication analysis for genes such as *BRCA1*, *BRCA2* and *MECP2* has demonstrated that such changes may be important sources of pathogenic mutations. Multiplex ligation-dependent probe amplification (MLPA) is a recent addition to routine DNA diagnostics allowing detection of copy gains or losses in an efficient, low-cost, robust way, with a high sensitivity and specificity. MLPA-kits have become available commercially for several genes with known deletions, including those mentioned above. As we expected that exon deletions and duplications would be an important source of pathogenic changes in other genes as well, we have developed a system in which we can rapidly implement MLPA-analysis for any gene desired by the use of fully synthetic probes. Briefly, 5 control probes with different lengths have been designed to be included in all kits. Additional gene-specific synthetic probes can be added to up to 15 fragments in total, each fragment 4 nucleotides longer than the previous one. To date, we have been successful for the autosomal genes *CHD7* , *EYA1*, *LMX1B*, *EHMT1,* and *SPG4*, and X-linked *CHM*, and *NDP*. Preliminary data indicate that indeed 1-5% of mutations in the autosomal genes is an exonic deletion or insertion. For the X-linked disorders, it allowed us the easy diagnosis of female carriers with a deletion.

P1207. Analysis of MOB (TMEM 23) expression in rats under brain experimental ischemia

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It is now well known that ceramide and diacylglycerol (DAG) are signal molecules of apoptotic and antiapoptotic pathways of cell regulation. Being element of sphingomyelin cycle sphingomyelin synthase 1 (SMS1) encoded the MOB (TMEM 23) major transcript controls the synthesis of ceramide and diacylglycerol which relative concentrations are responsible for balance between cell death and surviving. It is established that apoptosis is involved in mechanisms of cerebral ischemia. To investigate the expression of MOB major transcript in the ischemic brain we took advantage of experimental models of global and focal ischemia in rats. We used semiquantitative reverse transcription polimerase chain reaction (RT-PCR) to assess the mRNA level of MOB in the forebrain and cerebral cortex of rats in the permanent bilateral common carotid artery occlusion model of global ischemia and in the permanent middle cerebral artery occlusion (pMCAO) model of focal ischemia. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control. In the global ischemia model the expression of MOB major transcript was significantly decreased only after 24 h in forebrain cortex of ischemic rats compared to sham-operated and control animals. In the lesioned cortex of pMCAO animals MOB mRNA level decreased after 24 h compared to control group. At 48 h after pMCAO the expression of MOB major transcript has tend to increase in both lesion cortex and contralateral cortex of ischemic rats. In addition MOB mRNA level at 48 h after pMCAO was significantly higher compared to 24 h after pMCAO and control animals.

P1208. An evaluation of automated mutation detection using SoftGenetics® sequence data analysis software Mutation Surveyor™ v2.51

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In order to asses the effectiveness of automated mutation detection of Mutation Surveyor™ in a diagnostic setting, we tested four sets of bi-directional sequence data comprising 5.2Mb in total. The data covered a broad spectrum of sequencing chemistries, laboratories, sequencing platforms and read lengths.

In bi-directional mode, Mutation Surveyor™ is claimed to detect >99% of mutations, with sensitivity to the mutant allele extending down to 5% of the primary peak provided sequence quality meets a minimum Phred score of 20.

After excluding all possible explanations for false negative results following visual inspection of the trace data, the bi-directional false negative rate ranged from 0.0-4.9% depending on data set. Sensitivity was depressed for mosaic mutations and only 62% (33/53) were detected without visual inspection under default settings. Mutation Surveyor™ showed decreased sensitivity and an increased false positive rate on data produced by the Beckman CEQ8000 platform using the CEQ-DTCS chemistry.

Mutation Surveyor™ was able to de-convolute 89% (155/175) of heterozygote indel mutations into separate alleles. However separation into the two alleles did not permit the automated detection of mutations downstream of the indel, moreover the software did have difficulty in naming frameshift mutations sequenced in the reverse orientation.

Mutation Surveyor™ can make a significant contribution in helping to ease the burden of sequence data analysis. Although we have highlighted weaknesses with the program (used in auto-run mode with default settings), the user has the facility to increase sensitivity by altering many of the parameters.

P1209. Rapid and sensitive nanoparticle based assay for gene expression studies

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Gold nanoparticles have long been used as biological tags, and more recently, advances in functionalising particles with oligonucleotides have allowed for the development of a series of new and practical biodetection systems. Gold nanoparticle probes have arisen as novel tools for specific nucleic acid detection and can be used in biomolecule detection assays with numerous advantages when compared to more common conventional approaches (1).

In the present work, oligonucleotide-gold nanoparticle conjugates were employed to enable selective colorimetric discrimination of the β-globin gene (HBB) expression. The gold nanoparticle surface is functionalised with thiolated oligonucleotides (Au-nanoprobe), and in solution typically exhibits a red colour due to the optical absorption peak around 526 nm caused by surface plasmon resonance. High salt concentration induces Au-nanoprobe aggregation in the absence of a complementary sequence and the solution turns purple (absorption peak shifts towards longer wavelength); if the probe hybridises specifically to the complementary target sequence, there is no Au-nanoprobe aggregation and the solution remains red. The abundance of the specific mRNA was correlated with values of absorption at 526nm of a solution containing the probes and total RNA extracted from MEL cells previously transfected with the HBB gene. This gold-nanoprobe method proved to be sensitive, selective and extremely easy to perform, taking less than 15 minutes to develop after total RNA extraction, without the need for expensive and time consuming experimental set-ups.

(1) Baptista P., et al. 2005. Colorimetric Detection of Eukariotic Gene Expression with DNA-derivatized Gold Nanoparticles. Journal of Biotechnology, 119(2): 111-117.

P1210. *FINDbase*: A relational database for frequencies of inherited disease-causing mutations in different populations worldwide

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The National and Ethnic Mutation Databases (NMDBs) are continuously updated mutation depositories, recording extensive information over the described genetic heterogeneity of an ethnic group or population. Those resources not only enhance awareness over the various genetic disorders but also facilitate the provision of genetic services, by better orientating mutation screening, and the elaboration of the demographic history of human population groups. Here, we report the construction of *FINDbase* (http://www.findbase.org), a relational database, aiming at recording the frequency of mutations, leading to inherited disorders in various populations worldwide. Database operates under PHP and MySQL, is based on data warehousing and provides a simple, web-based, and extendable system for population-based mutation data collection. In addition, this database significantly contributes towards NMDB uniformity, as it allows the setup of several NMDBs locally and/or centrally, using a single database framework. Database records can be queried using a user-friendly query interface, providing instant access to the list and frequencies of the different mutations, accompanied by links to the respective Online Mendelian Inheritance in Man (OMIM) entries. Query outputs can be either in a table or graph format, accompanied by reference(s) on the data source. Registered users from three different groups, namely administrator, coordinator and curator, are responsible for database curation and/or data entry/correction online via a password-protected interface. Database access is free of charge and there are no registration requirements for data query. This database can serve as a valuable online tool for molecular genetic testing of inherited disorders.

P1211. The Lebanese National Mutation frequency database

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The exponential discovery rate of new genomic alterations, leading to genetic disorders, as well as the need for comparative studies of different populations mutation frequencies necessitates recording their population-wide spectrum, in mutation databases. The National Mutation frequency databases are continuously updated mutation depositories, which contain extensive information over the described genetic heterogeneity of a population or ethnic group. Here, we report the construction of the Lebanese National Mutation frequency database (http://www.goldenhelix.org/lebanese), derived from an academic effort to provide high quality and up-to-date information on the underlying genetic heterogeneity of inherited disorders in the Lebanese population. Database core engine has been built and maintained online using the specialized *ETHNOS* software (Patrinos et al., *Hum Mutat*, 2005; 25:327-333, Kleanthous et al., *Hum Mutat* 2006; in press) and contains brief summaries of the various genetic disorders prevalent in Lebanon and studied for the Lebanese population, namely beta-thalassemia, cystic fibrosis, 21-hydroxylase deficiency, GJB2 sensorineural deafness and familial Meditteranean fever. Additionally, an easy-to-use query interface provides instant access to the list and frequencies of the different mutations responsible for the inherited disorders in the Lebanese population. Furthermore, numerous links to the respective Online Mendelian Inheritance in Man (OMIM) entries and, where available to various locus-specific databases, fruitfully integrate the database's content into a single web site. This database can serve as a valuable online tool for molecular genetic testing of inherited disorders in Lebanon and could potentially motivate further investigations of yet unknown genetic diseases in the Lebanese population.

P1212. Non-enzymatic labeling of nucleic acids using The Universal Linkage System (ULS™) in gene expression DNA Microarray applications

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The Universal Linkage System (ULS™) is a (platinum-based) labeling technology to label RNA, DNA and proteins Since ULS labeling is a chemical labeling technology, complete control over the labeling process has been achieved. The “no need for enzymes” approach together with a controlled labeling makes the ULS labeling procedure very reproducible and robust.

The ULS labeling technology’s key advantage is the ability to directly label nucleic acids in their natural form. For example, ULS is very efficient at directly labeling naturally occurring small RNA/miRNA in a one step 15 min reaction. Other labeling technologies encounter issues trying to label these small (20-23 nts) non coding RNAs, thus making ULS a very attractive labeling technology in this field.

For gene expression DNA microarray applications, the available amount of mRNA can be limiting. For this reason, linear target amplification to generate aRNA is typically required. Traditionally, labeling of aRNA is achieved by introducing modified nucleotides during the linear amplification step. This is not ideal since it can reduce the efficiency of the enzyme and introduce bias. ULS offers the possibility to label aRNA generated with unmodified nucleotides, allowing the enzyme to work to its maximum efficiency. Avoiding the use of modified nucleotides gives much higher yields and better size distribution of aRNA.

Here data will be presented where the ULS labeling technology was evaluated on several gene expression microarrays platforms. Furthermore, results will be reported where the ULS labeling technology was evaluated for labeling and microarray detection of microRNAs.

P1213. High-throughput and comprehensive CHIP-based resequencing of the mitochondrial DNA in patients with OXPHOS disease

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Mitochondrial disorders are often fatal multisystem disorders, associated with abnormalities of oxidative phosphorylation (OXPHOS). Because of its dual genetic control, defects in OXPHOS can be due to mutations in either the mitochondrial (mtDNA) or nuclear DNA. Although OXPHOS disorders have common characteristics, there is considerable clinical variability among patients, even in those having the same genetic defect. Also, clinically indiscernible conditions can be caused by different mutations in a number of genes. Therefore, we applied a new CHIP-based platform for rapid resequencing of the mtDNA. We found a complete match with classical resequencing in 3 samples. Two samples were mixed to generate artificially heteroplasmy at 11 positions in the mtDNA sequence. Ten were detectable as such and one gave a no-call. Analysis of mixed amounts of different mtDNAs indicated that a mutation load of ≥10% should be detectable. Although the platform is not optimal for the detection of small deletions or insertions, we were able to identify two samples with a single nucleotide insertion as a heterozygote call. Screening of 20 patients suspected for mitochondrial disease revealed 171 different mutations: 90 SNPs, 5 known pathogenic mutations and 71 unknown variants (5 of which were heteroplasmic). Our conclusion is that the resequencing CHIPs are a very promising tool for rapid and comprehensive mtDNA-screening. Especially for genomes like the mtDNA, where it is possible to generate the template with a single PCR, and in which the vast majority of the point mutations are nucleotide substitutions, it will become the method of choice.

P1214. PeroxisomeDB: a database for peroxisomal proteome, genes and diseases.

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Peroxisomes are ubiquitously distributed organelles, exclusive from eukaryotic cells and organisms, essential for lipid metabolism and free- radical detoxification. Loss or malfunction of peroxisomes causes dramatic inherited conditions such as Zellweger syndrome or X-linked Adrenoleukodystrophy. We have created a peroxisomal database http://www.peroxisomedb.org with the purpose of gathering and clarifying all relevant information on peroxisomal genes, proteins, metabolic pathways and diseases, combining molecular biology and genetics, genomics, metabolism and clinical points of view. Using annotated data derived from online resources and the literature the peroxisomeDB includes the complete peroxisomal proteome of *Homo sapiens* (82 genes) and *Saccharomyces cerevisiae* (56 genes). The database is structured in interrelated sections ‘Genes’, ‘Functions’ ‘Metabolic pathways’ and ‘Diseases’ and includes hyperlinks to other databases (NCBI, ENSEMBL, UCSC, SNPs…). The proteins are classified into 51 different characterized metabolic functions. Interactive graphical depictions of the main peroxisomal metabolic routes and updated flow charts for diagnosis are included. The disease catalogue lists a total of 23 disorders sorted by clinical criteria and linked to reference databases such as OMIM, The Human Gene Mutation Database (HGMD) and disease-specific databases. Precomputed BLAST, PSI-BLAST, multiple sequence alignment (CLUSTALW) and phylogenetic trees are provided for each human entry allowing automated identification of orthologues and spotting of the principal domains conserved throughout evolution. We present a powerful tool for unequivocal *in silico* peroxisome identification, based on the BLAST search of four peroxisomal markers: Pex3, Pex10, Pex12 and Pex19. This will be particularly useful for screening new genomes in the search for the organelle.

P1215. The evolutionary origin of peroxisomes: an ER-peroxisome connection.

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The peroxisome is an essential eukaryotic organelle, crucial for lipid metabolism and free radical detoxification, development, differentiation and morphogenesis from yeasts to humans. Loss of peroxisomes invariably leads to fatal peroxisome biogenesis disorders in man (PBD). The evolutionary origin of peroxisomes remains unsolved; proposals for either a symbiogenetic or cellular membrane invagination events are unconclusive. To address this question, we have probed with a peroxisomal proteome, an *ensemble* of 19 representative eukaryotic complete genomes. Molecular phylogenetic and sequence comparison tools allowed us to identify 4 proteins as peroxisomal markers for unequivocal *in silico* peroxisome detection. We have then detected the Apicomplexa phylum as a first group of organisms devoid of peroxisomes, in the presence of mitochondria. Finally, we deliver evidence against a prokaryotic ancestor of peroxisomes: a) The peroxisomal membrane is composed of purely eukaryotic bricks and is thus useful to trace the eukaryotes in their evolutionary paths; b) The peroxisomal matrix protein import system shares mechanistic similarities with the endoplasmic reticulum/proteasome degradation process (ERAD), indicating a common evolutionary history.

P1216. Unattended screening of genetic polymorphisms by automating microfluidic on-chip electrophoresis

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In the “post-genome era”, there is an ever-growing demand for high-throughput DNA analyses. Especially in the context of genotyping and screening for genetic polymorphisms, the number of samples that are processed are steadily increasing, representing a major challenge for classical DNA analysis techniques. Especially slab gel and PAGE analysis require long analysis times, are labor intensive and difficult to automate.

Here, we present a new automated microfluidic system that allows the unattended separation, sizing and quantitation of hundreds of DNA samples. The system has been successfully tested to screen for a set of different genetic polymorphisms as deletions, insertions and

dinucleotide repeats identified in different target genes. Our results clearly show that sensitivity and sizing accuracy are superior to slab gel analysis and the digital data generated is highly reproducible. Due to the automation of all relevant analysis steps, the system is particularly suitable for transferring human genetic projects from the stage of target identification towards the high throughput screening approaches including the screening of thousands of patient samples.

P1217. Pooled DNA genotyping on Affymetrix SNP genotyping arrays

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It is now technically feasible to perform genome-wide association studies of complex diseases with hundreds of thousands of SNPs. However, the cost of such projects remains high. Pooled DNA genotyping offers the possibility of applying the same technologies at a fraction of the cost, and there is some evidence that certain ultra-high throughput platforms also perform with an acceptable accuracy. Until now this conclusion was based upon published data concerning only a small number of SNPs.

In the current study we prepared DNA pools from the parents and from the offspring of the 30 parent-child trios that have been extensively genotyped by the HapMap project. We analysed the two pools with Affymertix 10K Xba 142 2.0 Arrays. The availability of the HapMap data allowed us to validate the performance of 6843 SNPs for which we had both complete individual and pooled genotyping data.

Pooled analysis averaged over 5-6 microarrays resulted in highly reproducible results. The average error of predicting the differences in allele frequency between the two pools was 1.37%, and 95% of SNPs showed an error of < 3.2%. This provides a sufficient accuracy for case-control association studies of complex disorders.

It remains to be seen if this high quality will be reproduced on the Affymetrix 500K arrays, which would allow the rapid and affordable conduction of full-genome association studies.

P1218. Evaluation of MS-MLPA for molecular diagnosis of Prader-Willi and Angelman syndrome in a clinical context

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Prader-Willi (PWS) and Angelman (AS) are distinct syndromes characterized by developmental impairment, caused by loss of expression of imprinted genes in the paternal (PWS) or maternal (AS) chromosomal region 15q11-q13. Molecular genetic testing is important to confirm the clinical diagnosis and to predict the familial recurrence risk which depends on the underlying molecular mechanism [deletion (in 75% of PWS and AS cases), uniparental disomy (UPD), an imprinting error or point mutations].

In most diagnostic laboratories methylation-specific PCR or Southern blot analysis is used. Additional testing (FISH, UPD-analysis) is required to reveal the underlying molecular mechanism. An ideal molecular test should provide information about both parent-specific methylation imprinting on 15q11-q13 and the underlying genetic mechanism. Recently, methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) became commercially available. This technique allows detection of changes in CpG methylation and copy number of chromosomal sequences in a single reaction.

We evaluated this technique for diagnostic applications in a clinical setting. Using MS-MLPA we tested a group of 16 patients with previously confirmed PWS (7) and AS (9) respectively. The molecular diagnosis and underlying molecular mechanism were confirmed in all cases. A comparison was made between MS-MLPA and Southern blot results. We conclude that MS-MLPA is a powerful technique, suitable for molecular diagnostics of PWS/AS in a clinical context. MS-MLPA has important advantages compared to other techniques as starting from genomic DNA, information about methylation status and underlying molecular mechanism is provided within in a single experiment, resulting in a shorter turnover time.

P1219. TaqMan Preamplification for Real-Time Gene Expression Analysis with Sample Limited Specimens

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We have developed a robust solution for uniform amplification of cDNA prior to quantitative, real-time PCR. TaqMan® Preamp Master Mix allows up to 100 gene targets to be pre-amplified simultaneously using TaqMan® Gene Expression Assays as the source of pooled gene-specific primers. By incorporating a preamplificaion step into the sample preparation workflow, adverse sample splitting effects on binominal sampling are significantly reduced or eliminated. TaqMan® PreAmp Master Mix and optimized cycling parameters enable nearly 100% efficient amplification of target sequences. Benchmarking studies comparing TaqMan® preamplification to existing methods show that the TaqMan® preamplification method retains the relative copy numbers of starting targets more reproducibly and precisely. Also, the fold-amplification attainable with TaqMan® preamplification is greater. Data obtained from the Applied Biosystems 7900 Sequence Detection System demonstrate the wide utility of this process in many gene expression arenas including the profiling of cells obtained by laser capture micro dissection . Results using the TaqMan® preamplification of random-primed cDNA are independent of amplicon distance from the 3‘ end and are amenable to partially degraded RNA samples.

P1220. Microfluidic detection of a point mutation in the prothrombin gene by on-chip electrophoresis

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Prothrombin plays a key role in blood clotting. A single nucleotide polymorphism (SNP), i.e. a point mutation in the prothrombin gene, results in a common hereditary predisposition to venous thrombosis. The G20210A mutation in the untranslated part of the prothrombin gene causes elevated serum prothrombin level and an increased risk for venous thrombosis. Individuals heterozygous for the prothrombin G20210A mutation have a two-to three-fold increased risk for venous thrombosis and an elevated prothrombin serum level. A fast and easily adaptable method, suitable for the workflow at any standard molecular diagnostic laboratory, was recently developed. This convenient and reliable mutation detection is based on a PCR and a subsequent restriction digest (PCR-RFLP). DNA fragments are separated and sized by a commercial microfluidic system. The overall performance achieved with this technique is far superior to the conventional agarose gel electrophoresis in terms of sizing accuracy, quantitation capability, reproducibility and resolution. These findings suggest that this technique could also be adopted and easily used for further SNP detection assays.

P1221. qBase: relative quantification software for management and automated analysis of real-time quantitative PCR data

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Gene expression analysis is becoming increasingly important in biological research and clinical decision making, with qPCR becoming the method of choice for expression profiling of selected genes. Accurate and straightforward mathematical and statistical analysis of the raw data, as well as the management of large data sets remain major hurdles in qPCR based gene expression analysis. Since the software provided with the different detection systems usually does not offer an adequate solution for these issues, we developed qBase, a free program for the management and automated analysis of qPCR data.

qBase is a collection of Microsoft Excel sheets with VBA-code and uses a proven delta-Ct relative quantification model with PCR efficiency correction and multiple reference gene normalization. The qBase Browser allows data storage and annotation by hierarchically organizing qPCR measurements into projects, experiments, and runs. An import wizard allows easy import of export files from many currently available qPCR instrument softwares. The browser enhances easy access to all your data, and exchange of data between users. The qBase Analyzer converts Ct values into normalized and rescaled relative quantities with proper error propagation. It contains an easy run editor, performs numerous quality controls and inter-run calibration, and displays results both tabulated and in graphic format. The program

allows large numbers of samples and genes, variable number of replicates, and multiple runs to be processed together. The possibility to use up to 5 reference genes allows reliable and robust normalization of gene expression levels. qBase is freely available for download at http://medgen.ugent.be/qbase/.

P1222. A package of programs for quality control of genetic data

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In large data sets obtained in genetic studies the presence of errors is almost inevitable. Errors may result from imperfect nature of the process of the entry of the data to the database (e.g. typing errors), wrongly specified genealogical relations (e.g. mispaternity) and experimental error (e.g. genotyping and phenotyping errors). Accurate geno- and phenotypic information is crucial for gene mapping. Both pheno- and genotyping errors can have large impact on results of linkage and association studies leading to false negative or positive finding.

We developed a package of programs for testing of pedigree structure and detection of pheno- and genotyping errors. At the first step of the quality control procedure, the initial data on pedigree structure (*recode_ped.pl*), genotypes (*recodeSNP.pl, affy2mega.pl*) and phenotypes (*phenotypicQC.pl*) are transformed from arbitrary to the standard genetic format. Additional correctness of pedigree structure and consistency of phenotypic data is verified. At the second step, pedigree and genotypic data are combined using program *pre_pedcheck.pl*. The resulting file enters the test for Mendelian inconsistency, which allows identification of genotyping errors. At this stage, the external program PedCheck (O'Connell and Weeks, 1998) is used to detect errors of inheritance of autosomal markers and our program *x_check.pl* is used for X-linked markers. At the last stage, the information on errors is extracted from the output, connected to the initial coding and reported in table format. The programs can handle large data sets including tens of thousands of individuals, SNPs and polymorphic markers. The package is available at http://mga.bionet.nsc.ru/nlru/

P1223. 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 dependable PCR-sequencing primers for different genomic targets and with user-defined parameters would greatly facilitate resequencing.

We have developed an integrated web-based tool which incorporates target sequence selection/submission, 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(tm) 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. The generated primer information is formatted as a tab-delimited file so it can be easily uploaded into oligo vendors' ordering sites. Template files for automated discovery of variants compatible with Seqscape® software are also generated. Utilization of this primer design tool can substantially reduce the effort required to design and optimize robust resequencing primers.

P1224. Design, validation and public release of resequencing primer sets for over 15,000 human genes for analyses of sequence variation and SNP discovery

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Increasing attention has been focused upon SNP discovery and genotyping in an effort to associate disease/phenotypes with variations and mutations, and to determine evolutionary relationships, but reliable primer design and data analysis are among the major challenges.

A high-throughput, high-quality computational pipeline has been developed to design PCR primers for resequencing. Primer design is driven by the primer3 program and supplementary algorithms developed at Applied Biosystems. All known SNPs in the genomic sequence are avoided during primer design. The resulting primers are QA'ed by two computational tools: the first one checks the primer pairs for genomic redundancy using a proprietary version of the e-PCR program; the second tool, developed using a lab-validated training set from 200K amplicons, predicts the probability of success of the primer pairs. Project Template files are generated to allow integrated data analysis with the SeqScape® software.

We have attempted designing resequencing sets for all 16334 human genes with RefSeq mRNAs. Annotation data from NCBI and Celera have been combined to define the structure of these genes. We have achieved average coverage of 92% of all bases (coding exons, UTRs and 1kb upstream) and 94% within the coding regions. On average 21 amplicons are required to sequence a whole gene and 13 to sequence just the coding region. About 800 amplicons were lab-tested, with an overall success rate over 95%.

To promote the resequencing research, we have deposited the complete designs to the NCBI probeDB, and supplemental information will be freely available at AB's web site.

P1225. New approach for whole-genome identification of interspersed repeats insertional polymorphisms

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A new technique for genome-wide detection of integration sites of polymorphic retroelements (REs) is described. The technique allows one to reveal the absence of a retroelement in an individual genome provided that this retroelement is present in at least one of several other genomes under comparison. Since many genomes can be compared simultaneously, the search for insertions of polymorphic retroelements is very efficient. The technique includes two whole genome selective PCR amplifications of sequences flanking retroelements: one for a particular genome and another one for a mixture of about ten different genomes. A subsequent subtractive hybridization of the obtained amplicons with DNA of the particular genome as driver results in isolation of polymorphic insertions. The technique was successfully applied for identification of 41 new polymorphic human Alu Ya5 and Ya8 insertions. Among them, 18 individual Alu elements first sequenced in this work were found to be absent from the available human genome databases. This result suggests that significant part of polymorphic REs were not identified during genome sequencing and remain to be detected and characterized. The proposed method does not depend on preliminary knowledge of evolutionary history of retroelements and can be applied for identification of insertion/deletion polymorphic markers in genomes of different species.

P1226. Miniaturization of Liquid Handling Procedures in High Throughput Sequencing at the Broad Institute

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Deerac Fluidics™ Equator™ products are designed to fit a wide range of applications requiring low volume liquid handling. The products have recently been installed as part of the high throughput sequencing production process at the Broad Institute in Boston, MA, USA (formerly the Whitehead Center for Genome Research). The Genome Sequencing and Analysis program at the Broad Institute places emphasis on the

development of scalable methods employing automated laboratory procedures and informatics systems. The current rate of sequencing corresponds to over 40 million lanes per year, deployed in sequencing the human, mouse, and other genomes.
This poster will describe the current process employed by Broad Institute, highlighting where the Equator™ has played a vital role in performance enhancement and cost reduction, enabling the Broad Institute to retain its position as the world's leading genome research institute.

P1227. A simple and rapid purification method for generating high quality sequence data

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Removal of unincorporated dye-terminators from sequencing reactions and efficient desalting of these samples are crucial in ensuring maximum high quality basecalls and useable data. Applied Biosystems' new post-sequencing reaction purification kit, currently in development and testing, strives to not only ensure effective dye-terminator removal, but also provides flexible protocols to fit into all types of workflows and accommodates low to high throughput levels. Advantages of this novel method include a simple protocol with minimum hands-on time, improved sample stability, efficient desalting for electrokinetic injection, better recovery of smaller DNA fragments and efficient removal of unincorporated dye-terminators, all crucial to sequencing applications such as resequencing and de novo sequencing. In addition, this method performs directly in the sequencing plate, with no additional plates necessary for capillary electrophoresis. Here, we describe a method that simplifies sequencing workflow allowing you more time to focus on your research instead of sample processing

P1228. A system for effective and informative quality control of DNA sequencing in a clinical laboratory

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Obtaining consistent high quality data is critical to the effective use of DNA sequencing in a clinical laboratory environment and can only be obtained if appropriate approaches to quality control are implemented. Consistent high quality data reduces repeat rates and improves the accuracy of automated base calling. We have developed a flexible and unique QC system that is integrated into our Assign-ATF software that provides an effective and informative QC analysis of sequence data. Assign-ATF calculates a quantitative base call score (BSC) for each base call within a sequence generated by automated DNA sequencers. The BCS is influenced by sequence peak shape, the level of background and the degree of which a peak overlaps with neighbouring peaks. The mean BCS can be calculated for an entire sequence from a single sequencing primer resulting in a single quantitative quality value for a sequence. Similarly a BCS can be calculated for all sequences from a specific sample producing a quantitative quality value for a sequence. Assign-ATF can automatically produce longitudinal plots of BCS for all samples. Such a graphs produce quality control information essential for the maintenance of a clinical laboratory test. Such data includes
1 Sample to sample and sequence run to run variability of sequence quality
2 Rapid identification of changes systematic changes in quality
3 Setting of performance criteria for the evaluation of new reagents and protocols
4 Identification of factors that affect DNA quality
5 Assessment of interlaboratory concordance and agreement of appropriate sequence quality

P1229. Spinal Muscular Atrophy (SMA) genotyping by gene dosage using Multiple Ligation-dependent Probe Amplification (MLPA)

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Spinal Muscular Atrophy (SMA) is an autosomal recessive disease 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. About 95% of SMA cases are caused by homozygous deletion of the SMN1 gene (5q13), or to its conversion to SMN2. The molecular diagnosis of this disease is usually carried out by a PCR-RFLP approach able to evidence the absence of both SMN1 copies. However, this approach is not able to identify heterozygous healthy carriers, which show a very high frequency in general population (1:50). In this study, we used the Multiple Ligation-dependent Probe Amplification (MLPA) approach for the molecular diagnosis of SMA in 19 affected patient and in 57 individuals at risk to be healthy carriers. This analysis detected the homozygous SMN1 absence in all the investigated cases, and allowed to discriminate between SMN1 deletion and conversion to SMN2. Moreover, MLPA analysis evidenced a condition of heterozygous SMN1 absence in 33 out of the 57 subjetcs at risk to be healthy carriers. MLPA analysis represents an easy, low cost and high troughput system in the molecular diagnosis of SMA, both in affected patients and in healthy carriers.

P1230. Incorporation of nanolitre pipetting technology into medium and high throughput SNP genotyping platforms at KBiosciences.

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An area of great interest in drug discovery at present is the process of genotyping in order to better understand where genes, and in particular changes in genetic structure caused by single nucleotide polymorphisms (SNPs), can lead to the determination of targets associated with various disease states.
In some cases companies are actively pursuing genotyping processes in medium and high throughput fashion that will enable a large number of samples to be monitored in a short time frame and with minimal labour intensity.
The genotyping process can be costly to run at all levels, especially in the consumption of expensive reagents such as PCR mixes. spot-on™ nanolitre technology from Deerac Fluidics™ has been incorporated by UK genotyping services company KBiosciences into their high throughput system, which has enabled cost savings of up to 20 fold on previous techniques used.
This poster will describe the current process employed by KBiosciences highlighting in particular where spot-on™ technology has played a vital role in performance and reducing costs, which has enabled KBiosciences to become cost competitive in the marketplace in the provision of their service.

P1231. High through-put targeting induced mutations using Applied Biosystems' 3730 series capillary electrophoresis system

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Targeting induced mutations in genomes is a strategy used in reverse genetic studies to identify series of chemically induced point mutations in specific genes. The detection of point mutations exploits the ability of the CEL1 endonuclease to cleave genomic DNA at mismatched heteroduplexes. Polyacrylamide slab-gel electrophoresis systems are traditionally used to detect the cleavage products. These methods are labour intensive and not easily automated. We have developed the use of Applied Biosystems' 3730 series capillary electrophoresis system for cleavage product detection and sizing up to 1200 bp range in a 5-color dye-set. Following optimization of the CEL1 cleavage reaction, clean-up and capillary run conditions point mutations could be identified in populations with up to 12 fold pooling. For high through-put fragment analysis the AFLP Analysis Method in Applied Biosystems' GeneMapper® Software v4.0 was optimized for effective peak detection and enables accurate cleavage product sizing from 1Kb target regions. We present our progress in this method in a range of organisms and discuss strategies for the development of an automated processing system as well as alternative methods for high through-put detection of induced mutations.

P1232. Identification of transcription factor binding sites regulating myogenic differentiation by computer-assisted empirical promoter analysis

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We have developed a methodology to identify new transcription factors (TFs) involved in the control of myogenesis. We presumed these TFs can be found by searching for their functional binding sites in promoters of genes differentially regulated during myogenesis. *In silico* identification of TF binding sites (TFBSs) is difficult due to their small size (e.g. 6-8 bp) and variation among consensus binding sites, resulting in many false positives. To reduce the number of false positives we only considered homologs with similar regulation in different species and looked for evolutionary conserved regulatory sequences. Micro-array data sets from differentiating human and mouse myoblasts, generated in our and other labs, were queried with Perl scripts and the UniGene and HomoloGene databases. We found 14 homologs down-regulated and 30 homologs up-regulated in both species. The promoters of these human and mouse genes were obtained from the CSHLmpd2 database. These were aligned to recognition sequences of vertebrate TFBSs. With this procedure, we identified TFBSs that were found at significantly higher frequencies in promoters of genes differentially expressed during myogenesis than in genes with constant expression. We are now experimentally verifying these *in silico* findings using ChIP-(chromatin immunoprecipitation)-on-chip with various arrays. The combination of *in silico* and empirical approaches will assist in the identification of TFs with a role in the regulation of myogenic differentiation.

Po09. Genetic counselling, education, genetic services, and public policy

P1233. Genetic Counselling in neurological practice in Republic of Moldova

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Since the first introduction of genetic counselling services approximately 40 years ago many attempts have been made to devise a satisfactory and all-embracing definition.

In Scientific Research Institute of Mother and Child Health Service have been introduced the genetic counselling in neurological department. We note that frequency of hereditary diseases of neurological system has been increasing for last 5 years. . The families with increased risk of hereditary disease passed the three most important steps in genetic counselling: the establishment of a diagnosis, estimation of recurrence risk and communication of relevant information in a sympathetic manner.

The most crucial steps in any medical practice are establishing the diagnosis, and of cause the special diagnosis such as Sd. Dubowitz, Lissencephaly Sindrome, Sd. Menkes, Sd. Bardet-Biedl, oculo-auriculo-vertebral dysplosia etc. be able to establish only the medic-genetic. These introduction will allow important changes in the principals of the medical consulting: due to the DNA diagnosis, the neurologists have the possibility to detect the carriers of the mutant gene and in some cases to start the early treatment, in the presymptomatic phase of the disease (MDD, Chorcot-Marie -Tooth desease, Wilson Desease) . Plus, the DNA prenatal diagnosis allows esteeming the genetic status of the fetus and helps the prophylaxis of the repeated disease cases in the affected family (MDD, SMA, PCU).

Thus, the genetic counselling will allow changing the thinking of the neurologist and will dictate the introduction of the modern medical genetics counselling services (molecular genetic achievements) in the everyday practice

P1234. Molecular study of non-deletional alpha globin genes mutation among Iranian; reporting three novel mutations

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Thalassemia, as the most common monogenic disorder in the malaria-prone belt of the world, is still one of the social, economical, physical and mental problems of communities. Having various ethnic groups and suffering from inadequate knowledge of α-thalassemia mutations, in particular non-deletional mutations, Iran is one of the Middle East countries with high incidence of both types of thalassemia (α&β) that encounters major challenges in PND and pre-marriage counselling. Herein we tested 50 PND candidate samples, presenting low MCV & MCH, normal HbA2 levels and no deletional α-thalassemia mutations. These non-deletional α_{2}-globin gene mutations were analyzed by ARMS assay. Direct sequencing was administered on the samples that didn't reveal any gross deletional or non-deletional mutations. ARMS primers of -5nt, cd_{19} (reprted from Iran), cd_{142} and PA-2 mutations were designed and multiplexed for rapid detection. Direct sequencing was administered on the recovered specific α_{1}& α_{2} PCR products. -5nt & PA-2 (AAT<u>A</u>AA>AAT<u>G</u>AA) were the most frequent affected allele defined by ARMS-PCR & direct sequencing respectively. Also three novel mutations were detected so far; a frame shift mutation (-C) on the α_{1}-globin gene and two single base substitutions (G>T) on different codons of the α_{2}-globin gene that created a displaced stop codon. Pre-marriage genetic counselling and prenatal diagnosis centers for β-thalassemia require prompt, precise and perfect identification of suspected α-thalassemia carriers. The robustness and reliability of ARMS-PCR and its efficient use in β-thalassemia mutation detection has potentially led the method to become a popular method for α-thalassemia mutation detection.

P1235. Gendia Foundation. Helping to subsidize the cost of genetic testing for patients and families around the world

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The GENDIA Foundation is a non-profit organization, located in the United States, working to help subsidize the cost of genetic testing for people who cannot afford genetic services. The ultimate objective of the GENDIA Foundation is to facilitate genetic testing on a global scale.

A major bottleneck in genetic testing is the cost of services, which are often times unaffordable when not reimbursed by insurance companies. Several decades of intensive research on the genetic causes of human genetic diseases have resulted in a large list of tests that can be offered to diagnose genetic diseases. In many countries, laboratories have been established that offer genetic tests to patients and their families.

Unfortunately, there are huge differences with respect to accessibility, price and quality of the genetic testing in the various countries. The spectrum of genetic diseases tested varies from several hundreds in countries with a well-developed service system to only a few in most countries of the world. Moreover, the high prices of many genetic tests are unaffordable to many people when not reimbursed by insurance companies.

The GENDIA foundation was founded to support genetic testing of those that cannot afford it.

The foundation is a non-profit organization that raises funds to pay for genetic tests in selected cases. The GENDIA foundation also offers general information and support on genetics, genetic diseases and genetic tests.

P1236. The prevalence of false results for Thalassemia testing reported by peripheral labs in Tehran from 2002 to 2005

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Background: Beta thalassemia, characterized by the deficiency or absence of beta globulin production, is one of the most widespread inherited disorders in the world. In Iran, the prevalence of beta thalassemia trait (minor) is high, approximately 5-10%. In order to prevent beta thalassemia major, couples are screened for beta thalassemia trait before their marriage. Sometimes, in repeated premarital screening tests, significant differences between the recorded results for the first and second samples are found. For this reason, we decided to estimate the prevalence of incorrect laboratory

results for this screening test that are reported to patients in our region. Methods: The results of mean corpuscular volume (MCV) and HbA2 assays performed in peripheral labs and those from the assays repeated in the referral lab were compared to each other and to each patient's phenotype. Results: The comparisons indicated that 5% of primary reports for beta thalassemia trait from peripheral labs were incorrect. Half of these were false positive and half were false negative. Conclusion: Hematology laboratories in Iran should reconsider the efficacy of their instruments, their employees' skills, the precision of reported results and the quality of their lab management as a whole.

P1237. Prenatal Diagnosis of Single Gene Disorders: Iranian Experience

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This article provides an account of the introduction of molecular genetic testing initiated by prenatal diagnosis of Thalassemia, which has grown to a more comprehensive prevention program for genetic disorders in Iran.Molecular genetic testing in Iran was initially introduced in 1992 through performing prenatal diagnosis (PND) of β-thalassemia. This acted as a catalyst for thalassemia prevention program. When we started doing PND inside the country, the need to provide PND as the ultimate preventive measure was evident. However, the legal and administrative frameworks to deal with the aftermath of such a diagnosis; i.e. **abortion therapy** were not in place yet. Nevertheless, β-thalassemia with over 15000 registered major cases, many intermedia cases, estimate of three million carriers, expensive treatments and stigmas and psychosocial problems was so great that it could not be ignored. This defect was finally rectified in 1997 and the offer of PND to the at-risk couples was incorporated in the thalassemia prevention program run by the Health Ministry. Adoption of such a policy has paved the way for a more comprehensive approach in prevention of genetic disorders in general. Carrier testing and PND tests have now been made available for muscular dystrophies, hemophiliac and other genetic conditions. The byproduct of such activities have been training of specialists and technicians in molecular diagnosis, laboratory facilities and equipment in private and state sectors and collaboration between different specialists with clinical molecular geneticists. This article elaborates on preventive aspects of clinical genetics in the large and populous country of Iran.

P1238. The results of questioning of doctors on bioethics points

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We have conducted a social investigation on bioethics points of 200 neurologists, family doctors by means of specially compiled questionnaire. 99 percents of the examined stated the necessity of medico-genetic consultations for family members with the hereditary diseases, 70 % for pregnant women and 74% answered, that it is needed only after marriage. Prenatal diagnostics is believed by 82% of respondents as necessary , when mother reaches the age of 35. The detection of hereditary abnormality in fetus could lead to abortion by 90 % of respondents. Both parents "opinion (73%) is regarded by our respondent as the main having weight reason for making such a decision in case of hereditary pathology, 28% are always ready to place responsibility of making decision on the consulting doctor.

In most doctors" opinion (88%) DNA-testing on exiting mutations in genes is necessary and recommended to a healthy person, to that suspecting the existence of gene violations because of relatives" diseases (48%), to the sick, wishing to find out the nature of his disease (31%) and healthy people, who reached 18 (28%).

The wish to entirely posses the information concerning their health from 85 % of doctors prevailed over some possible negative consequences of the DNA-testing results (stress, depression and so on). The information concerning DNA-testing should be strictly confident as regarded by 98 % of respondents. This for the improvement of the specialist preparing in the field of medical genetics the introduction of bioethics points into programm of study in universities is required.

P1239. Social and ethical issues in research using human embryos: a contribution to the GeneExpress design study

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The University of Newcastle is coordinating the EU funded *GeneExpress* project; a Design Study that aims to evaluate the most effective ways of overcoming challenges faced by scientists involved in the analysis of gene expression in early human development. One strand of this project will explore the social, legal and ethical issues associated with such research, and this is being undertaken by the Policy Ethics And Life Sciences research institute (PEALS); a bioethics "think tank" based within the University, whose aims to are to research, inform and improve policy, professional practice and public participation in the life sciences.

Cultural and social attitudes to research using early human tissue vary significantly across the EU and so the ethical framework for such work needs to be carefully considered. Thus, alongside the scientific components of *GeneExpress*, PEALS will:

• Map the current situation by conducting a review of the social science and bioethics literature in this area.
• Systematically review legislation and regulatory guidelines in place for working with human developmental samples throughout the EU.
• Explore the requirements for governance and ethics training.
• Address the policy implications for continued ethical research in this field.

Research methods will include qualitative analysis of surveys and in-depth interviews with scientists active in research on human development. PEALS will also organise a forum in which a critical exchange of ideas can take place, with a view to developing a governance framework for future research. A longer term aim will be to include the views of a wider, non-specialist, public.

P1240. Reproducibility and validity of the Claus-Extended Formula in a British cohort of women with a family history of breast cancer

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Background. Risk estimation in breast cancer families is often performed using the Claus Tables. Previously, we constructed a new risk estimation method: the Claus-Extended Formula. This uses the Claus Tables (CT) and information on the presence of bilateral breast cancer (BBC), ovarian cancer (OC), and multiple breast cancer cases (MC) in the family: 0.08 + 0.40*CT + 0.07*OC + 0.08*BBC + 0.07*MC.

Aim. To validate the Claus-Extended Formula using a British cohort of families with breast and/or ovarian cancer.

Methods. We analysed 2156 family histories from a British Family History Clinic. We estimated lifetime risks of breast cancer using the Claus Model, the Jonker Model, the Claus Tables and the Claus-Extended Formula and considered correlations and agreements. Furthermore, we calibrated the Claus-Extended Formula in order to evaluate whether this Formula estimates the risks accurately in this other cohort.

Results. The British counsellees had on average 1.7 breast cancer cases per family (SD 0.8; range 0-6). Spearman Correlations between the Claus-Extended Formula and the Jonker Model, the Claus Model and the Claus Tables were 0.768, 0.679, and 0.770, respectively. Agreements were 73%, 33%, and 63%, respectively. The calibration of the formula showed no clinically relevant differences.

Conclusion. We found that the Claus-Extended Formula provides accurate lifetime risks of breast cancer, compared to estimates by the Claus model and the Jonker model. The Formula is easily applied in clinical practice. We, therefore, conclude that the Claus-Extended Formula is a valuable risk estimation method for clinical practice, both inside and outside the Netherlands.

P1241. Genetic testing in Italy, year 2004

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Italy is the only European country which has settled a comprensive and long range monitoring of genetic testing on a nation-wide basis, starting form 1987. The data collected by the last census of year 2004, on the behalf of the Italian Society of Human Genetics included the activities of 88 clinical centres, 160 cytogenetic and 183 molecular genetic laboratories, hosted by 256 structures, 16% of which were private. Only 42% of them fulfilled the requirements of current Italian legislation. Genetic tests included 283,601 cytogenetic analyses. There had been 120,238 invasive prenatal samplings, 84% of which were amniocenteses. This study has also surveyed 190,610 molecular genetic tests. On the total 420 different genes have been investigated, 10 of which accounted for three quarter of all this activity. In general, the demand of genetic tests has increased by a figure of about 10% each year, starting form 1997. Only 16% of cytogenetic and 12.5 of molecular tests have been accompanied by genetic counseling. This survey remarks the need of some basic intervention in the general organisation of the genetic structures in Italy, which should be rationalised, in respect of the national guidelines, and the need of continuing training of the general practitioner and education of the consumer to the appropriate use of genetic testing.

P1242. Chondrodysplasia puncata - a family case report

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Chondrodysplasia punctata (CDPX) is a condition which includes a clinically diverse group of bone and cartilage dysplasias which cause characteristic epiphyseal stippling. The inheritance is X - linked dominant. The molecular basis of this condition is the mutation in the emopamil-binding protein gene EBP (CDPX2) μαππεδ ον Xp11.23-p11.22.

CDPX2 patients display skin defects including linear or whorled atrophic and pigmentary lesions, striated hyperkeratosis, coarse lusterless hair and alopecia, cataracts; skeletal abnormalities including short stature, rhizomelic shortening of the limbs, epiphyseal stippling, and craniofacial defects.

We describe a family case, with three female family members affected with chondrodysplasia punctata. Results for EBP-screening showed that all affected family members exhibit a point mutation (substitutes the glutamine at the position 158 by a stop codon: pQ158X) in the exon 5 of the EBP - gene (c472C>T).

In this paper we discuss complex psychological issues in the three generations of the family affected with Chondrodysplasia punctata.

P1243. Towards measurement of clinical validity and utility of genetic testing in Europe

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In recent years a great deal of attention has been paid at the national and international level to develop policies in the field of genetic service provision. Eurogentest aims at addressing the challenges through an European Network of Excellence (NoE) in genetic testing by involving experts from across Europe and developing infrastructure, resources, guidelines and procedures that will structure, harmonise and improve the overall quality of all European genetic services.

We examined access to and uptake of as well as funding policies and costs of genetic testing in 6 European countries and received responses from presidents of human genetics societies from following countries with populations ranging from 5 to 80 million: Finland, Sweden, Portugal, UK, France and Germany.

The comparison between these countries indicates differences and similarities, such as a similar increase of DNA-based testing in Germany and the UK from 1999 to 2002 despite considerable differences in system regulation. In Sweden, DNA diagnostic and PND cytogenetic testing raised from 1996 to 2003, with PND testing increasing by a factor of 1.5. Whereas approximately 21,000 DNA-based tests per year are performed in Finland with a population of 5 m, only about 12,000 are performed in Portugal with a population of 10 m and at a relatively high price level in comparison with other countries.

There is as yet no consensus within the scientific how to measure clinical validity and clinical utility of genetic testing. Therefore further investigations are strongly needed and standards are to be developed to give general guidance.

P1244. Consanguinity among the Israeli Arab community: Is the pattern changing?

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In the Israeli Arab community the prevalence of consanguinity is relatively high, which is associated with high rates of inherited disorders leading to high frequency of morbidity and mortality in this community. Data on consanguinity between couples were received during two periods (1980-1985 and 2000-2004) in relation to socio-economic status in four selected villages.Two of them (A and B) are known to have a high socio-economic status, and the other two (C and D) are known to have a low socio-economic status. The incidence of consanguineous marriages in the four studied villages slightly decreased from 33.08% in the first period to 25.92% in the second period. On the other hand, the marriages within the first cousins showed a more significant decrease from 23.86% in the first period to 13.62% in the second period. The averages of consanguinity rate of the two villages (A and B) during the two periods were found to be 22.31% and 16.16% respectively, while those of the other two villages (C and D) during the two periods were found to be 42.33% and 37.22% respectively. There has been a change in the pattern of consanguinity in the selected villages of the Israeli Arab community during the two study periods. This change was largely affected by the socio-demographic status of the villages. Therefore, improving the socio-economic status of the villages, as well as, implementation of proper health educational programs are expected to have a positive effect in reducing consanguinity.

P1245. Etiological and consanguinity profiles of disorders referred to a genetic counseling clinic in Jordan

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With 25% of all marriages occurring between first cousins, increasing attention in Jordan is now given to role of consanguinity in the occurrence of genetic diseases. An inaccurate message addressed to the population on the disadvantages of consanguinity could lead to family and social disruption. The aim of this study is to elucidate the relationship of consanguinity to the variable etiological categories of genetic disorders among 550 families seen at the genetic clinic of the National Center for Diabetes, Endocrinology and Genetics in Amman over a period of 30 months.

Results: Autosomal recessive and dominant inheritances were the underlying etiology in 35% and 15% of cases respectively. A proportion of 33% of all cases remained undiagnosed, 82% of which were sporadic. First cousin marriage rates among parents of probands with autosomal recessive, autosomal dominant, X-linked , chromosomal and undiagnosed disorders were 67%, 26%, 28%, 31% and 49% respectively.

Conclusion: The rate of first cousin marriages was higher among families with autosomal recessive conditions and non-diagnosed conditions than the rate among the general population in the same area. In a community where selective termination of an affected fetus has its religious restrictions , prospective genetic counseling to families with segregating recessive genes and premarital testing and screening whenever feasible remain important measures for prevention and control of genetic diseases. On the other hand, consanguinity was not associated with other etiological categories. Messages to the general population regarding consanguinity should be properly phrased according to evidence based criteria.

P1246. Cystic fibrosis (CF) neonatal screening in the Czech Republic: results of a pilot study

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According to epidemiologic / genetic studies the incidence of CF in our country is 1:2700 newborns. Thus, given the current birthrate ~35 new cases of CF should annually be detected. However, registry data demonstrated that 1/3 of CF patients remains clinically undiagnosed and that the age at diagnosis has markedly increased (prior to 1998 median: 0.58; between 1999-2005: 1.2 years). Therefore, in II/2006 we started two tier (IRT/DNA) pilot CF neonatal screening project (NSCF) comprising Bohemian regions, representing ~2/3 of our population. Altogether 45,453 newborns were examined during the initial 9 month period. In 545 cases (1.2%), who had IRT concentration above the continuously adjusted cut off level, we examined the most common *CFTR* mutations. The diagnosis of CF was established in 5 newborns (2x F508del/F508del; 2x F508del/G551D; 1x F508del/R117H-7T - mild CF) and these children were subsequently referred to CF Centres. Furthermore, we detected 42 newborns with 1 CFTR allele (35x F508del, 2x CFTRdele2.3/21kb/, 2x N1303K, 1x G551D, 1x I507del and 1x I148T). Until now 27 follow up sweat tests (ST) were performed (26x <30mmol/L, 1x 30-40mmol/L). IRT recall was carried out in 14 children with IRT >200ng/mL and with a detectable CFTR allele - 11 children had negative IRT recall and 1 child was positive (ST negative). From these results the incidence of CF can preliminarily be adjusted to 1:9090 newborns. However, these results can be skewed due to lower number of newborns tested, effect of prenatal diagnosis and/or false negativity of NSCF. Supported by MZCR 8236-3, 00000064203.

P1247. Validation of a synthetic quality control sample in a European EQA scheme

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It is of great importance for public health that results from genetic services are correct because genetic tests for an individual are usually carried out only once in a lifetime. In order to assure high quality and accuracy of laboratory testing, appropriate control materials are essential. Laboratories mainly use commercially available cell lines and patient samples as controls. For many genetic tests, however, control materials are difficult to find. Based on this unmet need, synthetic quality control (QC) samples are being developed. Such synthetic samples can contain rare mutations, allow many mutations to be detected in one multiplex test and can be designed to function in distinct extraction methods.

MMQCI has constructed synthetic quality control blood samples for Cystic Fibrosis (CF), containing 27 *CFTR* exons including intronic borders, and carrying different mutations. A thorough evaluation of this sample was performed in house by MMQCI for several DNA extraction and PCR-based amplification methods, as well as different mutation analysis methods. In order to assess the value of this synthetic blood sample on a large scale, the European Thematic CF network enclosed it as an additional sample in their External Quality Assessment scheme of 2005.

More than 150 laboratories, in 46 countries registered to participate in the validation study of this QC sample. The outcome of this study will identify correlations between the detection of mutations and specific analysis methods used in routine practice. Furthermore, this validation should enable assessment of the usefulness and effectiveness of synthetic quality controls for CF testing.

P1248. The importance of the dental exam for identification and diagnosis of genetic diseases

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Genetic pathology often determines a complex and extremely polymorphic clinical phnotype. Among the frequently found anomalies are those in the dentomaxillary field. The Genetic Department from ,,Louis Turcanu" Children's Emergency Hospital investigated and observed between 2000-2005, 540 children with different genetic diseases. We find that 78% of them presented minor or major dentomaxillofacial anomalies. Among the registrated cases cromosomal syndromes, monogenic diseases, and multifactorial plurimalformative syndromes were found. The dentistry exam was often indispensable for the correct dentomaxillar anomalies diagnosis and highly important, revealing hardly detectable diseases. The early dental exam is necessary in all genetic syndroms, for a correct topic and adequate therapeutical directions. The dentist completes the multidisciplinary team which participates to diagnosis and observing genetic diseases.

P1249. The role of team work in social reinsertion for Down syndrome children

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Introduction: In the absence of a governmental strategy for Down syndrome, the most common chromosomal disease in Romania, the social inclusion of these children remains to the private initiative of NGO's.

Aim of our paper is to focus on the changes in life quality of children with this syndrome, after a multidisciplinary approach.

Material: In our NGO partnership (a branch of an international NGO from Romania and a local one) we worked with 32 children and youngsters affected by 21 trisomy. They participate from 1999 in a Down Club organized by young volunteers from our organizations.

Results: Children with Down syndrome have interacted with volunteers and benefited by becoming more assertive and by achieving more developmental milestones. Assessment of their progress might establish the exact role of communication among the Down Club. The parents have witnessed important cognitive and behavioral changes in their children, facts that at this moment are our only method of evaluating their progress.

Conclusions: The health of people with disability and the social integration can be improved if they have every opportunity to enjoy family life, education, friendship, access to public facilities and freedom of movement. Action should be aimed at counteracting helplessness and stigmatization.

Developing awareness about the needs of children with Down syndrome and engaging public in a shared strategy for the development of genetic services, based on WHO principles ,,health for all in the 21st century" will ensure a collaborative international approach in sharing of expertise and experience.

P1250. Evaluation of informativeness of microsatellite markers for carrier testing of dystrophic epidermolysis bullosa in Tunisia

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Dystrophic Epidermolysis Bullosa (DEB) is a clinically heterogenous blistering disorder of the skin and mucous membranes characterized by abnormalities in the anchoring fibrils (AF) and loss of dermal-epidermal adherence. It is inherited in either autosomal dominant or recessive mode. Mutations within the *COL7A1* gene (3p21) which encodes collagen VII, the major component of the AF, have been shown to be responsible for DEB. *COL7A1* is composed of 118 exons overlapping 32Kb. More than 200 mutations have been identified and most of them correspond to private mutations. Mutation screening is hampered by the size of the *COL7A1* gene and by the mutation heterogeneity. Analysis of polymorphic markers may provide a rapid and relatively easy method to identify carriers for genetic counselling.

Our aim is to evaluate the informativeness of polymorphic markers for carrier testing DEB in Tunisian families at risk. For this purpose, six consanguineous Tunisian families with at least one child affected have been analysed. Transmission of the disease within these families follows an autosomal recessive mode. All family members were

genotyped with five microsatellite markers overlapping the *COL7A1* gene. The genetic map of this region is: cen- D3S1568, D3S3629, (*COL7A1*), D3S643, D3S1478, D3S3582-tel. For the two closest markers to *COL7A1*, D3S643 was fully informative in the six families and D3S3629 was fully informative in 5 families and partially in 1 family. Taking into consideration the informativeness and distance of these 2 markers to *COL7A1*, we propose the use for carrier testing of DEB in North African population.

P1251. Genetics education for the nursing profession: identifying the needs of educators and practitioners.

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The NHS National Genetics Education and Development Centre nursing programme is using the genetics competency framework (Kirk et al. 2003) as the basis for education needs analyses of educators and practitioners.
UK higher education institutions teaching the nursing professions were invited to participate in a review of current genetics education provision [n=81; response rate 48%]. Using a questionnaire, respondents:

1. considered whether students on pre-registration courses were equipped to achieve the genetics competencies;
2. commented on the teaching methods and resources used;
3. identified what help they need in order to integrate each competency into their curricula.

The data indicate that there is notable variation in teaching levels across courses even within individual establishments. Although many of the competencies are currently not being achieved, respondents are positive about the role that the NGEDC can play in supporting their teaching.
An original approach is being used to canvass the views of non-genetic practitioners, many of whom see genetics as being unrelated to their role. A 'tear-out' questionnaire has been developed and piloted, for publication in six specialist nursing journals, each being immediately preceded by an article illustrating the relevance of genetics to patient care within that specialist group. It is anticipated that engaging individuals through their specialty and helping them to reflect on their practice in this way will enhance response rates.
This paper will report on how these studies are helping to direct resource development and promote genetic literacy across the profession.

P1252. Study on patients with ENT abnormalities among consanguineous marriages in Iran

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The high percentage of the patients which referred to the ENT clinics, has some kinds of congenital ENT abnormalities. Some of them are life threaten, the others cause disability and esthetic problems, and has profound consequences for the affected child and the family.
The ENT abnormalities usually place stress upon interpersonal relationships, social isolation, unhappiness and depression.
The majority of these abnormalities is genetic and follow AR inheritance.
The geneticists believe that consanguinity increase the probability of occurrence of AR disorders.
The aim of this study was to find out the frequency of all the ENT abnormalities among the consanguineous marriages.
We studied all the 3503 pedigrees of patients which referred to us for genetic counseling during the years 2003 and 2004.
From 3503 files, 206 files had ENT abnormalities, and out of them 157 files were with consanguineous marriages.
Among these 157 pedigrees 496 cases were the result of consanguineous marriages, and 219 cases were affected with ENT abnormalities.
Therefore, these 219 cases were studied according to consanguineous and non-consanguineous marriages, kind of relationships of the parents, patterns of inheritance, and sex, by using SPSS software.
Out of 496 cases, 4 abnormalities were the most frequent, consisted of: deafness 115 cases (23.2%), hearing loss 53 cases (10.7%), cleft lip palate 12 cases (2.4%), and isolated cleft palate 5 cases (1%).
Our results showed that, on the whole, there is a relationship between ENT abnormalities and consanguinity and the parents of the most affected individuals have consanguinity.

P1253. The European Skeletal Dysplasia Network; a four year review

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The European Skeletal Dysplasia Network (ESDN) was established in January 2002 with a grant from the European Commission and a remit to develop an integrated research and diagnostic network for skeletal dysplasias. Skeletal dysplasias are a diverse and complex group of rare genetic disorders affecting the development of the skeleton. Over 200 different conditions have been described, ranging in severity from mild to lethal. The overall prevalance of these conditions is at least 4 per 10,000, suggesting that at least 180,000 people in the 25 EU member states suffer from these bone diseases.
ESDN links centres of excellence involved in specialist research and/or diagnosis of skeletal dysplasias; Michael Briggs & Rob Elles (Manchester, UK); Geert Mortier (Ghent, Belgium); Michael Wright & Judith Goodship (Newcastle, UK); Jacky Bonaventure, Martine Le Merrer & Valerie Cormier-Daire (Paris, France); Leena Ala-Kokko & Minna Mannikko, (Oulu, Finland); Bernhard Zabel & Juergen Spranger, (Mainz, Germany); Andrea Superti-Furga & Sheila Unger (Frieburg, Germany) Luisa Bonafé, (Lausanne, Switzerland) and Christine Hall (London, UK).
Since January 2002, ESDN received approximately 2000 patient referrals and performed over 1500 molecular diagnostic tests. From September 2003 over 400 cases have been referred using the ESDN Case Manager; a secure web-based case management system that allows clinicians to submit cases directly to ESDN from anywhere in the world thereby enabling access to expert advice.
In summary, ESDN has successfully demonstrated it can act as a "virtual European centre of reference" for skeletal dysplasias by linking a multi-disciplinary group of clinicians, radiologists and scientists.

P1254. Knowledge and attitude of medical students toward ethical aspects of genetic counseling and selective abortion

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Genetic counselling is a communication process which deals with human problems associated with the occurrence, or the risk of occurrence of genetic disorder in a family. A primary goal of genetic selection is to increase capacity to diagnosis, treat and eliminate genetic disorders by selective abortion. In response to growing needs on genetics in medicine, it has been proposed that general practitioner should provide a frontline service in clinical genetics. According to importance and delicacy of these issues, present study evaluates the knowledge and attitude of medical students toward ethical aspects of genetic counseling and selective abortion before and after passing genetic course.
The results show the knowledge of medical students before passing genetic course is very low, and it is not increased enough after passing genetic course. However, they have right beliefs but mercilessness to abnormal foetus.
In conclusion, medical genetics progresses very fast and in the early future could eliminate some of genetic disorders, therefore education and justification of the medical staffs will be very important.

P1255. Ethical issues in genetic services in Islamic context

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The scientific and technical developments in human genetic research gave rise to a vast spectrum of ethical and societal believes. The consequences of the scientific findings on different aspects of the life, necessitates development of appropriate dealings with the relevant scientific findings and its outcome.
The Islamic communities hold strong and prevailing traditions within the framework of their faith, including the patterns of marriages, large family size and interlinked life style among family members. These encounter challenges of scientific applications in health field particularly those linked to the prevention of human genetic disorders. These situations recommended the need to establish framework of legal and ethical principles that govern the applications of the resultant information in diagnosis, prevention and health care.
This paper outlines the feature of the relevant ethical framework in the Islamic communities, taking into consideration religion, social and legal aspects in the broader term of ethical principles.

P1256. Production of Certified Reference Materials (CRMs) for the analysis of the human Factor II (prothrombin) gene G20210A mutation

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The availability of reference materials (RMs) for genetic testing is crucial to ensure that diagnostic laboratories can demonstrate the quality of the measurement services, and currently only few CRMs for clinical genetic testing are available. In collaboration with the Scientific Committee of Molecular Biology Techniques (C-MBT) in Clinical Chemistry of the IFCC, the European Commission- Joint Research Centre, Institute of Reference Materials and Measurements has developed, produced and characterised plasmid CRMs for the analysis of the G20210A mutation in the human prothrombin gene. A 609 bp gene fragment that spans all primer annealing sites published until today was selected. Both the wildtype and the G20210A mutated alleles of this gene fragment were cloned into the pUC18 plasmid and two plasmid RMs were produced. In addition, a third RM consisting of a mixture of both plasmids in equal quantities was produced, mimicking the heterozygous situation. The homogeneity, short-term and long-term stability of the three CRMs stored at different temperatures were analysed, showing stability of the material for more than 6 months at - 20 °C. Moreover, the fitness for purpose of these reference materials was investigated by 7 laboratories* with expertise in genetic testing, and the materials performed with excellence, thus being fit for purpose.
*Acknowledgements: Clinical Institute of Laboratory Diagnosis, Zagreb, HR; University of Pecs, H; Ctr. Study Haemostasis & Thrombosis, University of Ferrara, I; Ludwig-Maximilians-Universität, München, DE; Sheffield Haemophilia and Thrombosis Centre, UK; Allgemeines Krankenhaus Wien, Medizinische Fakultät der Universität Wien, A; Krankenhaus der Stadt Wien Lainz, A.

P1257. Bioethics, Genetic Counselling: Aspect of Theologians, Physicians and Geneticists in Iran

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Introduction: Genetic counselling sessions are rich and complex sites of accounting practices for decision-making in which clinicians are meant to facilitate rather than control the decisions made by their clients. Counsellors engaged in genetic counselling are ethically obligated to provide prospective parents with the basis for an informed decision for childbearing.
This study did for determining the attitude's professionals towards ethical issues in conducting of genetic counselling.
Material & methods: The group used questionnaire and face to face interview for data gathering. For data analysis, researchers applied the descriptive analysis.
Results: Eighty four percent agree with the parents' right to choose genetic counselling and screening. A majority of participants (83.3%) believe that counselling about performing screening decreases disability in the new generation. most of volunteers (71.0%) disagree with increasing an emotional trauma into mothers. About 52% of them said that counselling of the result of genetic screening, persuade mothers into terminating pregnancy and abortion before ensoulment period, but eighty one percent disagree that we don't use this diagnosis test because of failing fetus in prenatal screening. And, seventy four percent disagree that labeling individuals as being at risk of a disorder cause anxiety and invulnerability.
Conclusions: We face a positive attitude of all respondents to the benefits of genetic counselling. This study is part of a series in which we have attempted to gather and report the opinions of the scholars and religious thinkers in Iran regarding the bioethical considerations of genetic counselling.

P1258. Genetics education through patient-based scenarios: Raising 'genetic awareness' in the dietetics profession as part of a national genetics education strategy

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In the UK, dietitians work within the National Health Service (NHS) to provide dietary advice for patients, both in hospitals and in the community. For dietitians, relevant genetics includes understanding gene-environment interaction in a clinical setting. This is in contrast to the 'traditional' risk assessments for chromosomal and single gene disorders needed by most clinical health professionals.
The NHS National Genetics Education and Development Centre (NGEDC) has produced a strategy for integrating genetics across dietitians' career stages, including the development of competences for practicing dietitians to inform pre-registration training. Genetics 'champions' from the dietetics community met at NGEDC in December 2005 to discuss the present and future impact of genetics on dietitians and to develop a strategy for raising the profile of genetics within the profession.
Participants identified scientific advances in nutrient-gene interactions and nutrigenetic testing as key educational topics for the future. To ensure the relevance of genetics education and to ground education in clinical dietetic practice, patient-based scenarios were identified as an educational tool.
Small working groups are producing clinical scenarios on topics including obesity, coeliac disease, familial hypercholesterolaemia and interpretation of nutrigenetic test results. The scenarios will be interactive, with audience responses recorded using an electronic voting system. They will be presented at a dietitians' continuing professional development event in May 2006.
The questions posed during the clinical scenarios will provide feedback about the current genetics-related knowledge, skills and attitudes of UK dietitians and provide a platform on which to base future educational interventions.
www.geneticseducation.nhs.uk

P1259. Genetics and healthcare professional education in the UK: challenges identified by a national genetics education centre

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Two challenges are presented to healthcare professionals by advances in genetics - to use current clinical applications to provide a quality service for patients, and to understand and use future changes. The English Department of Health has established an NHS National Genetics Education and Development Centre to try to meet these challenges.

But before devising a programme of educational delivery it is important to identify and understand the drivers of and blocks to professional learning. For instance, NHS professional groups are at different stages in their acceptance and understanding of genetics, many appearing to be at the level of "unconscious incompetence". Mapping existing knowledge and skills, and determining attitudes are therefore helpful first steps in devising educational strategies which are responsive to the individual needs of different professional groups, the societal values in which they work and their ethos of learning. Initiatives also need to be grounded in clinical practice to demonstrate the relevance of genetics.
To accommodate different perspectives and learning styles, a range of resources are required. The NHS National Genetics Education and Development Centre website has therefore been organised to support those learning genetics, teaching genetics, developing genetics services, and applying genetics in practice by providing information on resources and learning support materials for each group.
As well as encouraging educational initiatives, an increasing role for genetics in mainstream medicine may have implications for service organisation and funding if competences in genetics are included in existing or new clinical roles.
www.geneticseducation.nhs.uk

P1260. Genetics Education- experience in a genetic service

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Medical Genetics department of University of Medicine and Pharmacy "Victor Babes" Timisoara and Genetics Department of Children Hospital "Louis Turcanu" have started several years ago a project which aim was to realize a rapprochement between genetics and community. Organizing courses and case presentations, including discussion of etiology, inheritance, dysmorphology, differential diagnosis of genetic diseases investigated and followed-up in Pediatric Clinics leads to a better addressability for genetic consult and a more rapid diagnostic, having a real benefit for the patients. Genetic Department has also the initiative of advisory teams organizing, focused on different specialties (ophthalmology, orthopedics, surgery, neuropsychiatry, psychology, cardiology, kinetotherapy, etc.). Referring patients to a specialist with a vast experience is very important for an accurate diagnostic and also for time saving. With family acceptance and presence, patient's consult was realized by entire multidisciplinary team. This kind of medical consult has a special impact on family members: they could discuss with all specialists in very short time, avoiding long hospitalization and multiple medical consults. Parents were very satisfied with the special attention accorded to their child and were able to understand the difficulties of an accurate diagnostic of rare genetic diseases. Increasing the confidence in medical team, good collaboration between geneticist, general physician, specialist and family represents the goal of our Genetic Department.

P1261. Attitudes and views of non-genetics trainee medical specialists towards training in genetics

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It is important to understand the views of trainees towards genetics in their clinical practice in order to tailor genetics education initiatives and ensure maximum effectiveness. The attitudes and views of non-genetics trainee medical specialists towards training in genetics were therefore explored through a survey using focus groups, questionnaires and interviews. Participants were 143 learners from four medical specialties (general practice, neurology, cardiology and dermatology) in two regions in England (West Midlands and South Western).
Low levels of specific training in genetics in their training programmes were reported by both trainee family practitioners and trainee hospital consultant specialists. With the exception of some cardiologists, the specialty trainees felt that there should be formal training in genetics. Overcrowding of the curriculum was an issue for trainees in all specialties.
All trainees stressed the importance of tailoring genetics education to be relevant to their daily clinical practice. Family practitioner trainees prioritised topics related to identifying and referring appropriate families, and the subsequent implication of results. This contrasts with specialty trainees who prioritised topics related to the genetics and management of particular diseases relevant to their specialty.
Knowledge of these views and attitudes will help in raising awareness of the relevance of genetics to non-genetics specialties' clinical practice and in ensuring that the genetics education provided is appropriate to the target group. Involvement of specialty trainers in the development and delivery of genetics education may help to address the issue of recognising the relevance of genetics.
www.geneticseducation.nhs.uk

P1262. Association between the hemochromatosis gene (HFE) mutations and rheumatic diseases.

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The objective of this study was to establish the frequency HFE gene mutation in the general population of the Czech Republic and among patients with hemochomatosis and with rheumatic diseases. Patients and methods: The HFE gene mutations (C282Y, H63D and S65C) were screened for by restriction enzyme analysis performed on PCR amplified products. These mutations have been assessed in 32 patients with hereditary hemochromatosis (HHC), 120 patients with polymyositis (PM) or dermatomyositis (DM), 246 patients with juvenile idiopathic arthritis (JIA), 184 patients with rheumatoid arthritis (RA), 94 patients with systemic lupus erythematosus (SLE), 120 patients with scleroderma (SCL), and in 481 control healthy persons. Results: Homozygous C282Y, H63D and S65C mutation was found in 90.6% of patients with hemochromatosis, which was significantly different from the the control group with 6.9 % C282Y heterozygotes, 26.6% H63D heterozygotes, and 2.5% S65C heterozygotes ($p<0.001$). Heterozygous C282Y mutation was found in 12.2% of patients with JIA ($p<0.05$). Insignificantly increased prevalence of H63D heterozygotes was found in patients with SCL (28.3%) and SLE (31.9%). Numerically higher representation of heterozygotes for S65C mutation was seen in patients with JIA (3.3%), RA (3.8%), and SLE (4.2%). No difference was observed in patiens with PM and DM. Conclusions: Heterozygous status for C282Y mutation may be a risk factor for juvenile idiopathic arthritis.
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P1263. Clinical genetics of the Hutterite Brethren: a European genetic isolate residing in North America

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The Hutterite Brethren are an Anabaptist sect living in North America. Although they arrived from the Ukraine, their origins were in the Tyrol region of Austria. All 30 000 Hutterites originate from less than 100 founders. Features of this population including founder effect, large families, meticulous records and high uptake of health care contribute to its suitability for genetic research. Over 30 autosomal recessive (AR) disorders have been described in this population (Innes et al, 1999). These include 'common' conditions such as cystic fibrosis (CF), limb-girdle muscular dystrophy 2I (LGMD2I) and spinal muscular atrophy. A large number of provisionally unique AR conditions have been identified. In many cases the responsible genes have been mapped or identified in collaboration with Hutterite families. Examples include *VLDLR*-associated cerebellar hypoplasia, AR Barth syndrome (*DNAJC19*), LGMD2H (*TRIM32*), Usher 1F (*PCDH15*) and Bowen-Conradi syndrome (12p13.3).One Hutterite CF mutation, M1101K, is rare worldwide, but has been reported in Tyrolean patients with CF. We anticipate that there should be evidence of other alleles that are enriched in the Hutterite Brethren in patients living in Central Europe. For example, a severe form of Joubert syndrome has recently been

reported independently in the Hutterites (Boycott et al, 2005) and in a Tyrolean family (Janecke et al, 2004).
This paper will review the clinical features and molecular basis of the AR disorders we have characterized in this population and will discuss the challenges and prospects for delivering genetic services to this population that has contributed significantly to genetic research.

P1264. Determinants of the intention to follow a salt-restricted diet in case of genetic vulnerability

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An important risk factor of heart diseases is hypertension. About 10% of the Dutch population suffers from hypertension. Due to genetic variation in salt sensitivity, the blood pressure between individuals varies as a result of salt intake. The aim of this study is to gain insights in the factors which determine the intention to follow a salt-restricted diet in case of genetic vulnerability.
Sixty-seven participants completed a written questionnaire, measuring demographics, social influences, attitudes, stages of change, risk perception, fear and danger control processes, and intention to follow a salt-restricted diet.
Half of the respondents had the intention to follow a salt-restricted diet in case of genetic vulnerability. Self-efficacy expectations and severity of the symptoms were highly related with the intention to follow a salt-restricted diet. About half of the respondents would engage in danger control processes (actions to prevent danger). Compared with 'fear controllers', 'danger controllers' had a higher intention to follow the salt-restricted diet, and were more often in the action phase. 'Danger controllers' were more convinced that salt-restricted diets might prevent hypertension, more often discussed genetic diseases with their friends, had higher self-efficacy expectations, were more convinced that knowing about their salt sensitivity would help them getting medical assistance, and that knowing about salt-sensitivity was in the interest of their children. Finally, 'danger controllers' were more normotensive.
The results of this study show that health education should focus on improving self-efficacy expectations and skills when promoting a salt-restricted diet in case of genetic vulnerability.

P1265. Uptake of predictive genetic testing in hypertrophic cardiomyopathy

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Introduction: Hypertrophic cardiomyopathy (HCM) is an autosomal dominant disease occasionally associated with sudden cardiac death (SCD) at young age if untreated. Genetic counselling and cascade-screening for HCM starts with a proband. After the detection of a pathogenic mutation the proband informs the relatives, by means of a family letter, about the aims and possibilities of predictive testing. Carriers of a disease-causing mutation are referred for cardiological screening and regular follow-up aiming at reduction of SCD.
Aim: This study focused on the family members' uptake of genetic counselling and predictive testing and the relationship between SCD in a first degree relatives and uptake.
Results: In 34 families with the 2373insG Dutch founder MYBPC3 gene mutation, on average 2.12 first degree relatives attended for genetic counselling in the first year, corresponding with 40.9% of all eligible first degree relatives. This number declines in more distant relatives (Table 1). All family members who attended genetic counselling proceeded with presymptomatic DNA-testing. There was a trend towards a higher uptake in families in which SCD had occurred (odds ratio 3.27, 95%CI: [0.68-15.82]).
Conclusion: Less than half of the eligible relatives opted for genetic counselling. Research into the determinants of the uptake, including the process of invitation, is needed.
Table 1: Average number of family members who attended genetic counselling in the first year by degree of relationship to the proband.

	First degree (n= 72)	Second degree (n= 31)	Third degree (n= 18)	Fourth degree (n= 3)	Total (n= 124)
Mean (SD)	2.12 (2.33)	0.92 (1.36)	0.53 (1.40)	0.09 (0.37)	3.65 (3.59)

P1266. Non-genetics trainee medical specialists: What genetics do they need to know for their clinical practice?

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Previous needs assessments with non-genetics medical specialty trainees have generated lists of genetic knowledge, skills and attitudes. In order to be useful for those involved in educating trainees, these must be organised into a simple, appropriate framework that can be targeted to different groups. The UK's NHS National Genetics Education and Development Centre's (NGEDC) initial work with medical professionals has therefore focused on developing core concepts in genetics for non-genetics trainee medical specialists and ways to make them relevant to their clinical practice.
Six broad genetics learning outcomes that medical trainees should attain by the end of specialist training have been identified. These were developed building on a web-based Delphi survey of a national sample of specialty consultants and consultant geneticists who identified key genetics knowledge, skills and attitudes required by non-genetics medical specialty trainees.
Geneticists have been consulted on these learning outcomes for delivering genetics education to non-genetics trainee medical specialists. They considered the learning outcomes and sub-topics to be applicable to all postgraduate medical specialties and agreed that they should be taught with reference to specialty-specific conditions in order to emphasise the relevance of genetics to clinical practice. A list of relevant conditions has been developed for cardiology, dermatology and neurology and work with other specialties is underway.
These learning outcomes provide a useful framework for geneticists involved in educating non-genetics medical specialty trainees, identifying educational priorities and conditions that can be used to ground core genetics concepts in clinical practice.
http://www.geneticseducation.nhs.uk

P1267. Organization of the medical-genetic assistance in Bashkortostan Republic of Russia

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In Bashkortostan Republic of Russia several stages health care system is established. At the first stage all the patients are investigated. The goal of the first stage is revealing of all cases with hereditary diseases and individuals at risk. The goal of the second stage is medical genetic counselling of families with hereditary diseases. As a result medical aid appealability for prognosis for health increased on 70%. The screening of newborn children for phenylketonuria is carried out from 1988 and for congenital thyreoid deficiency is carried out from 1993. The screening is held in 99% of all cases. Infant mortality in Bashkortostan Republic significantly decreased during the last 5 years on 32.9%. Mortality caused by congenital malformation decreased on 30% for the same period.

P1268. Exploring the current and anticipated role of genetic practice by midwives in Australia

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Developments in genetic diagnosis around pregnancy have affected not only parents, but also the health professionals that are involved in their care. In Victoria, Australia, midwives are an essential component of the prenatal and postnatal care of women, yet their role in genetics has not been described, recognised or supported. Using a qualitative approach we explored how genetics is incorporated into the current and anticipated role of midwives by conducting focus group discussions and semi-structured interviews. A total of nine focus groups were conducted with midwives (n=50) and 11 interviews with organisational managers and educators from seven maternity hospitals across Victoria. Two focus groups were also held with prenatal and neonatal genetic experts (n=10). Midwives discussed their experiences in dealing with genetic issues, while managers and experts were asked about their opinions on midwives' practice, and what they anticipate their role to entail. Transcripts of focus groups and interviews were analysed and major themes identified. Both managers and midwives recognised that midwives care primarily for normal or low risk pregnancies. Experts supported autonomy in regards to discussing prenatal and neonatal screening tests, whilst managers saw midwives essentially as resource and information providers. Midwives reported that their role in regards to genetics includes information provider, counsellor, and support person. Perceived boundaries with other health professionals was also discussed. Genetics appears to be a component of the midwife role through the whole continuum of pregnancy, with their role defined by organisational practice, patient demographics as well as personal experience and knowledge.

P1269. Genetic testing in families of patients with germline *MutYH* mutations: is it cost-effective?

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Introduction: MutYH associated polyposis (MAP) is an autosomal recessive disease, patients with bi-allelic germline mutations in the *MutYH* gene (MAP patients) are prone to develop between ten and hundreds of adenomas at a mean age of 45 years and in 60% of cases also colorectal carcinoma. In the general population about 1,5 % is a heterozygous *MutYH* mutation carrier. Children of *MutYH* mutation carriers have an increased risk of inheriting two *MutYH* mutations as compared to the general population and thereby an increased risk for colorectal carcinoma.

Methods: Using data from literature review and our own group of MAP-patients (n=40) we constructed a Markov model to perform a societal cost-utility analysis of family screening (testing children after the spouse has been tested positive).

Results: In absence of FOBT population screening, the cost-utility ratio of testing families of MAP patients, as compared to no genetic screening, was estimated at €19,000 per quality adjusted life year (QALY). The presence of FOBT population screening only slightly increased this cost-utility ratio to €20,000 per QALY. For testing families of heterozygote *MutYH* carriers, cost-utility ratios were about twice as high.

Conclusions: The costs per QALY for testing families of MAP patients are acceptable according to international standards. The conclusions of our analysis were sensitive to several of the parameters in the model, including the assumed €640 per genetic assessment.

P1270. The development of a personal health record for neurofibromatosis type 1

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Personal Health Records (PHRs) are client-held records. Their primary aim is to empower individuals and their families, giving them information about and ultimately more control over their health. PHRs exist for diabetes, heart disease, and cancer, as well as for antenatal care, and to monitor child growth and development, and these records have been positively received by both patients and clinicians. Our project involves developing PHRs for a range of common genetic conditions, beginning with Neurofibromatosis Type 1 (Nf1).

Nf1 is a relatively common autosomal genetic disorder, which is variable and unpredictable. There is a lifelong risk of developing serious complications, so patients need regular reviews and targeted screening at specific ages, and may be under the care of several specialists.

The PHR has been developed by a local consensus group, comprising; consultant geneticists, genetic counsellors and a Specialist Nf Advisor. The record contains; contact details, targeted background information, pages to record clinical details, pointers to further sources of information and support, as well as space for patients to make their own notes.

The Nf1 PHR will be piloted locally and its usefulness evaluated by questionnaires and focus groups. The Nf1 PHR has been designed to be as generic as possible, so it can be used as the basis for the other PHRs we intend to develop. In the long term, we hope that PHRs for individuals with genetic disorders will be recognised and accepted across all health care sectors as a gold standard in patient care.

P1271. Detection of glycine substitutions in the amino end of type I collagen by biochemical screening of fibroblast collagen requires supplementation by direct sequencing for osteogenesis imperfecta probands.

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The biochemical test for osteogenesis imperfecta detects structural abnormalities in the helical region of type I collagen as delayed electrophoretic migration of alpha chains synthesized by cultured fibroblasts on SDS-Urea-PAGE. The sensitivity of this test is based on overmodification of alpha chains in helices with a substitution of an invariant glycine residue in a Gly-X-Y triplet. Biochemical testing is most sensitive for structural changes in the carboxyl end of a chain because helices fold in the carboxyl to amino direction. Although the biochemical test has been used for 2 decades, the limits of detectability are unreported. We compared the electrophoretic migration of normal type I collagen with collagen from 30 OI patients (types III or IV) and known mutations in the amino half of the α1(I) and α2(I) chains. Sensitivity differed for each chain, was greater on 5% than 6% PAGE, and in intracellular than secreted collagen. In α1(I), substitutions in the first 100 residues were undetectable; 7% of cases in the current Mutation Consortium database are in this region. α1(I) substitutions between residues 100-230 were variably detectable while those after residue 232 were all detected. In α2(I), variability of detection extended through residue 436. About a third of cases in the Consortium database are located in the combined variable detection region. There was no correlation of substituting residues and biochemical sensitivity. Complete testing of probands with normal type I collagen biochemical results requires supplementation by direct sequencing of cDNA or gDNA in the amino regions of the alpha chains.

P1272. Paternity testing requested by private parties: ethical and deontological problems raised over the last few years

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This study regards the major problems encountered over the last few years by our Unit concerning requests for paternity testing by private parties.

Paternity testing based on DNA analysis have been adopted as the most important method, to objectively prove paternity legally and otherwise. It supplies information of great importance in order to satisfy the need for *biological truth*.

In the Italian judicial system biological proof is no longer understood to be an exceptional means of proof but is understood to be part and

parcel of investigations "which have probatory value equal to that of other proof". This has been the cause of a perceptible increase of not only judicial expert surveys but also extra judicial requests.
If, however, in the judicial arena, the laws today permit a clear framing of the cases in point in which it is possible to carry out an investigation without running the risk of abuse of or damage to other interested parties, there still exists no regulation of extra judicial investigation requests, nor is there any clear judicial opposition.We propose to identify the problem areas so as to aid in determining whether to accept investigation requests as made by private parties under various circumstances. This assumes greater importance in the private arena because, since there is less legal protection there, citizens have an even greater right to be protected.
The requests are evaluated and an attempt is made to highlight the typologies that may pose problems or reflections of an ethical nature.

P1273. An electronic preconception checklist on Internet: www.zwangerwijzer.nl

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During the last few decades perinatal and maternal mortality rates have stabilised or have even increased. The first trimester of the pregnancy is essential concerning the etiology of birth defects as well as disturbances in early placental development resulting in pregnancy complications in later pregnancy such as preeclampsia.
Initiating care *before* conception, preconception care, could therefore be the most effective strategy to improve the outcome of the pregnancy. Identification of risk factors is a main component of preconception care. Self-administered questionnaires have been proven to be accurate screening tools. We have adapted these questionnaires into an automated electronic checklist on the website, www.zwangerwijzer.nl. It is online since January 2004. It is being used by parents to be for their own information as well as a screening tool, facilitating the implementation of programs of preconception care in The Netherlands.
Zwangerwijzer focuses on identifying risk factors, supplies information about health promotion gives advise for additional preconception counselling in necessary. Zwangerwijzer had 42.157 visitors between June 1st, 2004 and May 25th 2005. Anonymous data are recorded after informed consent. 47% of visitors completed the whole questionnaires. Among female participants, 65,8% of them took folic acid supplements, 21,5% smoked and 16,9% took medication. Zwangerwijzer will make it possible to acquire risk profiles of subpopulations such as ethnic minorities. Although Internet has limitations as a scientific tool, we have found similar data as in other surveys. Zwangerwijzer will be an important instrument for the further implementation of preconception care in the whole of The Netherlands.

P1274. Premarriage Counseling

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Premariage Checkup Center In Nasser Institute Hospital (PCC-NIH)
Aimed Sector for the checkup are cases of:
1. Youth of both gender going to be married for full premarital checkup and genetic counseling.
2. Pregnant mothers that have a risky pregnancy to get babies with inherited diseases. To do prenatal diagnoses tests..
3. Couples had a child with inherited disease for diagnosis and treatment; and do genetic counseling for assessment of recurrence risk rate in next pregnancy.
4. Couples had general health problems that may affect the marriage for advice and treatment.
Our Mission:
1. Decrease the Handicapped Children rate in our country.
2. Decrease the rate of divorce in new families that has been reached 34.5%.
Our Goals:
Prevention
Education.

P1275. Carrier diagnostics and prevention of hemoglobinopathies in early pregnancy in The Netherlands: a pilot study

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We have offered, for the first time in The Netherlands, Hemoglobinopathies (HbP) carrier diagnostics to 139 randomly selected early pregnant women. The aim of this study was to establish whether carrier analysis would be welcome by the public and feasible at the outpatient level. Carrier diagnostics was accepted by 136 women (97.8%). The population consisted for 31% of recent immigrants and 69% of native Dutch. One carrier of HbS and one of β-thalassemia were found, both among the group of the recent immigrants. In both cases partners were controlled excluding a couple at risk. In addition, five carriers of α^+-thalassemia were diagnosed at the molecular level, one of them in the native Dutch population. Basic carrier analysis was done both at the Hospital Laboratory and at the Reference Laboratory. No discrepancies were found. This pilot study shows that 1) as predicted the prevalence of risk related HbP and of α^+-thalassemia is high in the immigrant population. 2) The compliance with carrier analysis in both native Dutch and immigrant is virtually total 3) Carrier diagnostic in early pregnancy and partner analysis in Hospital Laboratories is possible and is an effective tool for primary prevention of the Hemoglobinopathies in The Netherlands.

P1276. European proficiency testing study on the competence of laboratories to recognise rare mutations resulting in unexpected genotyping results

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Common genetic variations are tested using assays designed to detect a mutation of interest and not intended for mutation screening. However, SNPs close to this mutation have been reported to result in atypical genotyping results on some test systems. Testing laboratories should recognise these cases, to avoid misdiagnosis and inadequate treatment.
In order to assess the competence of testing laboratories to recognise and report such SNPs, four proficiency testing materials were processed for the analysis of the human factor II (prothrombin) gene G20210A mutation. Two of the four materials contained mutations giving atypical genotyping results using some methods.
These samples were sent to 283 laboratories through 3 EQA organisers, 189 from these laboratories participated to the study. Mutations C20209T and [T20175G+20179-80 del AC] resulted in atypical genotyping results in 65 and in 85 laboratories, respectively. Eighty-three (55.3 %) from these results were reported as one of the expected genotypes, 31 (20.7 %) were described as atypical results and only 36 (24 %) were recognised as another variant in the gene.
In the cases, when samples gave a typical result with the method used, the error rate was 4,7 %. Detailed results showed that more then 60 % of the false results were reported from only 8 laboratories. Furthermore, allele-specific amplification based PCR had a much higher error rate then other methods (16.7 % vs. 3.4 %).Results of this study indicated that majority of the false results could be prevented by improved training and careful selection of the method used.

P1277. Quality Assurance in European genetic laboratories

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Testing for genetic diseases has moved progressively from a predominantly research context into specialized clinical genetic laboratories. Concomitantly, there has been a greatly-increased attention to issues of quality control (QC) and assurance (QAu), particularly with respect to EQA and accreditation.

Existing initiatives collecting information on or providing support for QAu in genetic testing services are fragmented, and their continuity is not assured. The EU Network of Excellence EuroGentest is bringing together many initiatives to develop the necessary infrastructure, tools and resources to improve and harmonize the overall quality of genetic services throughout Europe.
Although a number of public websites provide lists of genetic testing laboratories and tests that are available, public information about QAu is sparse or absent. As a first step, we surveyed the current status of accreditation, certification and participation in EQA in European laboratories. The survey was distributed to more then 2000 contacts in 35 countries. To ensure the highest possible quality of the data, the collected information will be peer-reviewed prior to dissemination to laboratories and consumers via a European QAu database. The results will be presented.With the new awareness of the central role of QAu, making this information available will benefit consumers, by facilitating informed choice of laboratory partners for performing tests, and genetics services, by facilitating selection of partners for referral of tests which cannot be performed locally and by valorizing their efforts and investment in QAu. This survey will provide the first overview of the status of QAu in European genetics laboratories.

P1278. Quality Management and accreditation of genetic testing services

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Molecular genetic, cytogenetic and biochemical genetic services in Europe, while based on high quality scientific know-how, suffer from a high level of technical errors and poor reporting. In response to this, EuroGentest (EUGT) intends to structure and harmonize the overall quality of these services. Within EUGT, the Quality Management Unit will improve the organization and harmonization of EQA schemes, facilitate the development of guidelines, disseminate QAu (Quality assurance) information through a database and encourage services to attain and maintain accreditation. In order to assist laboratories preparing for accreditation, EUGT is also reviewing suitable quality control materials (QCM) and providing documentation or SOPs on new technologies.
Since EUGT was funded in January last year, progress has been made to disseminate information on accreditation requirements through the organization of two international workshops. EUGT distributed a survey to review the current status of QAu in European genetic testing services. Furthermore, research on the current situation of EQA providers and EQA schemes in Europe was carried out. Several forums for national and European EQA providers started discussing harmonization of EQA schemes and addressed the minimum quality standards for cytogenetics. A first European symposium was organized to set priorities for the development of QCMs. In order to start validation of several methods and technologies, a core service of accredited laboratories was set up and the first expert groups were established. All these initiatives will finally improve the quality of the management and provision of genetic services for the benefit of the patient.

P1279. Inheritance pattern in repeated pregnancy loss

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Introduction: pregnancy loss is the most common complication of pregnancy. About 1% to 2% of couples experience three or more consecutive spontaneous pregnancy loss, suggesting some underlying operative mechanisms. Among them genetic factors can include single gene defects as well as parental chromosome abnormalities and polygenic or multifactorial disorders.
Material and methods: 200 consecutive cases were studied by genetical analysis. Genetic counseling, clinical, paraclinical, and cytogenetic studies were done for each couple.
Results: There was positive familial pedigree of pregnancy loss in 176 couples (88%). They were divided by pedigree patterns in 4 groups:
1- RPL in 2-3 generations (12%)
2- ≥ 2 familial marriages with RPL (15%)
3- ≥1 other dispersed pregnancy loss in pedigree (61%)
4- Negative pedigree for pregnancy loss (12%)
There were other cases of RPL in 109 pedigrees (54.5%) from 1- 7. High rate of familial marriage was seen in involved couples (59%). Chromosomal abnormality was found in 15.8% of among 120 couples.
Conclusion: Previous investigations have demonstrated that the aberrant regulation of cellular process such as immunological, metabolic, vascular and endocrine may lead to PL and can be influenced by genetic factors. The presence of inherited disorders with low viability within a family can also be associated with RPL. Familial pedigree and inheritance pattern have a key role in genetic evaluation of couples with RPL.

P1280. Predictors of accuracy in recall of genetic cancer risk

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Genetic counseling for cancer aims to increase information about cancer and genetics, improve accuracy of risk perception, enhance informed decisions and risk management, and to help coping with threat of cancer. This study investigates predictors of accuracy in genetic risk recall with the objective of informing future counseling strategies. It is based on genetic counseling for cancer in two Cancer Society offices from April 2000 to February 2002. The counselor notified about the inherited cancer risk according to predefined categories of 'not increased', 'moderately increased', and 'markedly increased' risk.
Notified versus recalled genetic cancer risk was studied in a survey pre-counseling (T^0), and 2 (T^1) and 12 weeks (T^2) post-counseling (n=46). In addition to perceived/recalled risk, knowledge, perceptions, social support, mood, anxiety, dispositional optimism, health behavior, perceived health, sociodemographics, and family history plus other experience of cancer were measured. Five clients got not increased/ low risk notification at counseling; 21 moderately increased risk; and 20 markedly increased/high genetic risk. Based on recalled risk the subjects were divided into three groups: accurate recall (T1:n=26 / T2: n=20), under (n=16/19) and overstated risk (n=4/6). DISCRIM analysis was used to find factors best discriminating the groups. Both at T^1 and T^2 family history of cancer, optimism and social support were predictive of accurate risk recall, and at T^2 also higher better knowledge of genetics and cancer, and higher social status. Higher number of children was predictive of over statement at both time points: and better self-perceived health of under statement at T^1.

P1281. Aging in patients with Sanfilippo type B syndrome

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Sanfilippo B syndrome (mucopolysaccharidosis IIIB, MPS IIIB) is caused by deficiency of α-N-acetylglucosaminidase, a lysosomal enzyme involved in the degradation of heparan sulfate. Accumulation of the substrate in lysosomes lead to neurologic degeneration, behavioural problems, and mental decline. Compared to other types of MPS, somatic features are relatively mild in Sanfilippo B. It is the most common subtype of MPS in the Netherlands and probably underdiagnosed in adult persons with mental retardation.
We report the clinical data of 16 patients with Sanfilippo B. 13 of them derived from 2 large families and could be followed for several decades, and 3 were sporadic adult patients followed for a period of 10 years. 7 of 16 patients had died at ages 15 to 69 years, mainly from pneumonia and cachexia. The others were 37-62 years of age and all lived in institutions. Apart from the youngest, they had lost ambulancy,

at 36 to 62 years. Most had developed physical problems, in particular in the late 4th to 6th decade: cardiac disease (cardiomyopathy, atrial fibrillations), arthritis, skin blistering, swallowing difficulties requiring feeding by a percutaneous endoscopic gastrostomy tube, and seizures. Most of them showed or had shown restlessness and behavioural problems with hitting and extreme screaming which was difficult to prevent or to treat pharmaceutically. Adequate care and treatment plans of these challenging problems and of the medical complications will be illustrated.

P1282. Transition of medical care in persons with mental retardation: the Maastricht model

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Healthcare transition in an essential part of healthcare provision, referred to as the shift from one type of healthcare to another. Nowadays, many children with childhood onset diseases and/or genetic syndromes and/or mental retardation survive into adulthood. These children are at risk not being provided with coordinated and adequate healthcare as they reach adulthood.

With respect to healthcare transition, the paediatrician may hand over the care for the patient to a general practitioner. Hence, in case of any healthcare problems, the general practitioner usually will refer the patient to a (sub)specialist. This may result in many hospital visits to many different doctors. Fragmentation of the patients' healthcare may develop due to a lack of central coordination of care. For mentally retarded patients living in institutions, good coordinative healthcare is provided for these patients by a doctor for mentally retarded patients.

In Maastricht, the Netherlands, we developed an outpatients' ward. This ward is primarily aimed at providing healthcare coordination, when needed, for adult patients with an intellectual disability and/or a genetic syndrome. The prime goals of this outpatients' ward are coordination of healthcare, continuation of healthcare, providing good quality of healthcare and providing age- and developmentally appropriate healthcare. The consultations are held by a clinical geneticist and a doctor specialized in patients with an intellectual disability. Other (medical) specialists can be consulted. During the appointment, not only medical issues are discussed, but also psychosocial aspects concerning the patient. We present our first experiences with this model.

P1283. Initiatives to enhance the role of Public Health Care and Genetics on disease control

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Introduction: Public Health (PH) and Genetics are often considered as two independent subjects of health care. PH looks simultaneously at the control of epidemiological diseases and malnutrition in community-based populations which is a problem predominantly in developing countries. Meanwhile, in developed countries the increasing incidence of diseases such as cancer, diabetes etc. is considered a critical problem. While Genetics deals with the micro-level of health, PH looks at populations at a macro-level. However, both aim improving the quality of health and decreasing the morbidity and mortality.

Methods: Public Health Genetics (PHG) programs should be proposed and applied for each country. Analysing of PH and Genetics' data, identifies the diseases with highest incidence, possible causal or contributing factors, the aetiology and history of the diseases, relevant treatment protocols as well as cost of tests and therapies. Evidence-based results will suggest innovative preventive programs based on recent genetic findings. Important is the costs-analyse each disease-management, whereby the country-specific legal and ethical influences should not be overlooked. Country-based PHG strategies with innovative disease-management protocols should be proposed.

Discussion: PHG will propose innovative disease-management schemata. Application of cost-effective mechanisms will be possible; consequently, the population at risk will be identified and offered to undergo genetic screening tests, vaccination or therapy. Educational programs about PHG for the public and health professionals will change the health behaviour of people at risk and will provide them latest treatment by health professionals. Therefore, more diseases will be prevented and lower global mortality will be achieved.

P1284. The first case of genetic counseling for a compound heterozygous girl with Wolfram syndrome

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The first case of genetic counseling for a compound heterozygous girl with Wolfram syndrome.

Wolfram syndrome (WS) is a rare autosomal recessive neurodegenerative disorder characterized by diabetes insipidus, diabetes mellitus, optic atrophy, and deafness (DIDMOAD, OMIM #222300). This results in the majority of the cases from mutations in the WFS1 gene located on 4p16.1. The author reports on a girl with WS who presented with juvenile-onset diabetes mellitus when she was 9 9/12 years old. Optic atrophy was found at 10 years of age. No other abnormalities typical to WS were observed. Mutation analysis was performed by German colleagues. The proband was found to be compound heterozygote. 4bp deletion in exon 8 and 16bp deletion in exon 4 of the WFS1 gene were identified. Analysis in the parents has disclosed 4bp deletion in exon 8 to be paternal and 16 bp deletion in exon 4 to be maternal. Genetic counseling included consideration of problems of medical management and prognosis for the proband, for her parents and her semi sibling male twins.

Po10. Therapy for genetic disease

P1285. A novel strategy for β-globin gene targeting

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β thalassemia is one of the common autosomal recessive disorders. It is characterized by the reduced or absent of β-globin chain in affected ones. Transplantation of genetically corrected autologous human stem cell is an attractive approach to cure the disease. In this study we had developed a new strategy to correct the mutant gene by means of homologous recombination. A specific gene construct for β-globin gene targeting was designed and constructed. This construct consist of two homologous stems including 2.2 kb upstream (USHBG) and 2.5 kb downstream regions of β-globin gene (DSHBG), 2.1 kb β-globin gene (HBG) as the target gene, hygromycin, neomycine resistant genes as positive selection markers and thymidine kinase genes (TK1, TK2) as negative selection markers. All segments were amplified by PCR and cloned into pTZ57T/A cloning vector and then subcloned into pBGGT vector in the following order: TK1-USHBG-HYGROMYCIN-HBG-NEOMYCINE-DSHBG-TK2. This construct was linearized and transfected to cos-7 by lipofection. Positive and negative selections were preformed on these cells. Then PCR was preformed on DNA of the selected cells. The authenticity of cloning and subcloning steps was checked by PCR, restriction analysis and finally by sequencing. The results showed that only three clones of cells remained at the end of the selection. DNA of the selected cells was analyzed by PCR and sequencing regarding to homologous recombination. The result of sequencing confirmed the occurrence of homologous recombination in these cells. Therefore, a novel strategy gene replacement was done in one step and by using one construct.

P1286. Partial dystrophin correction in exons 30, 37 and 60 by gentamycin-induced translational readthrough

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Defective expression of the dystrophin gene leads to Duchenne muscular dystrophy (DMD), a severe X-linked neuromuscular disorder that is fatal by the second decade of life. About 15% of all DMD cases arise from premature stop codon mutations. Aminoglycoside-induced readthroughs of nonsense codons have been reported in several genes like *ATM* and *CFTR*. Our study investigates the use of gentamycin for dystrophin repair of nonsense codons in exons 30, 37 and 60 which were identified from our DMD patients. Cell extracts from the patients carrying UAG, UAA and UGA premature termination codons (PTCs) were treated with gentamycin at concentrations of 0, 2, 10, 20, 100, 1000 and 2,000 μg/ml for 2 hours for *in vitro* protein synthesis

assays and 48 hours for reporter expression. Restoration of reading frame was measured by mRNA analysis, *in vitro* protein synthesis and reporter expression. The treated cells exhibited varying levels of restoration with synthesis of corresponding dystrophin fragments of 52.9 kDa, 12.5 kDa and 52 kDa in relation to each of the three premature stop codons. These fragments correspond to the normal protein synthesized from the cDNA segments carrying the PTCs in exons 30, 37 and 60 respectively. Partial restoration of dystrophin translation was thus demonstrated from the *in vitro* studies in our DMD patients. It is believed that aminoglycosides can correct translation from nonsense codons by ribosomal interference and the complexity of readthrough regulation warrants further investigation since varying results have been previously reported from different studies using gentamycin to correct PTCs.

P1287. Fabry disease: Clinical manifestations in a cohort of 723 females.

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Fabry disease (FD) is a lysosomal storage disorder due to the deficiency of the lysosomal enzyme α -galactosidase A leading to severe multi-organ dysfunction and premature death in hemizygous males. Enzyme replacement therapy (agalsidase beta) has been shown to clear the vascular endothelial accumulation of glycosphingolipids and to decrease the incidence of significant clinical events in treated patients. Although the inheritance is classically considered as an X-linked recessive disease, it is increasingly apparent that heterozygous females may frequently present with features of the disease despite having measurable α -galactosidase A activity. The Fabry Registry (*www.FabryRegistry.com*) was analyzed to determine presenting symptoms, age at diagnosis, and age at clinical events in 723 heterozygous females for FD.

The median age at diagnosis was 33 yrs. (N=610; 24 yrs. in males). First symptoms were presented at a median age of 13.0 yrs. (N=344; 9.0 yrs. in males). At presentation, the most common symptoms were dermatological (18%), ophthalmological (19%), gastroenterological (20%) and pain (39%). Cardiac events (myocardial infarction, arrhythmia, angina, congestive heart failure or significant cardiac procedure) occurred in 18% at median age of 47 yrs. (24% in males). Cerebrovascular events (strokes) occurred in 8% at median age of 42 yrs. and renal events in only 3% at a median age of 38 yrs. (15% in males).

Whether Fabry disease should be considered as an X-linked dominant disorder needs additional data and follow-up. Awareness of the common presenting signs and symptoms in females may lead to improved recognition and outcome for symptomatic heterozygotes.

P1288. Treatment outcome of agalsidase alfa in Fabry disease

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Background Fabry disease is an X-linked lysosomal storage disorder caused by the absence or deficient activity of the lysosomal enzyme α-galactosidase A. The disorder is characterized by mulitsystemic disease that, untreated, progresses to multiple organ failure. The Fabry Outcome Survey (FOS) - an international database of patients with Fabry disease - was established to monitor the safety and outcome of enzyme replacement therapy (ERT) with agalsidase alfa in patients with Fabry disease.

Methods Data from FOS were analysed to determine the outcome of agalsidase alfa treatment on renal function, cardiac size, pain and health-related quality of life (HR-QoL).

Results Of the 752 patients enrolled in FOS at the time of the analysis, 436 (60% male) were receiving ERT, some 180 of whom had been treated for more than 3 years. Renal function, as assessed by estimating glomerular filtration rate (GFR) using the modification of diet in renal disease method, remained stable for the duration of treatment in patients with an estimated GFR between 30 and 90 ml/min/1.73 m^2. Significant regression of left ventricular hypertrophy (p<0.05) was demonstrated with ERT, with a mean reduction of 15-20 $g/m^{2.7}$ in left ventricular mass in the second year. This was accompanied by a significant improvement in cardiac function (midwall fractional shortening) after 1 and 2 years of treatment (p<0.05). Significant improvements in HR-QoL (p<0.05) and neuropathic pain (p<0.05) were also demonstrated with ERT.

Conclusion Analysis of FOS clearly demonstrates the clinical benefits of long-term ERT with agalsidase alfa in patients with Fabry disease.

P1289. Life expectancy in type 1 (non-neuronopathic) Gaucher disease

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Objective: Investigations were conducted to estimate life expectancy at birth of patients with type 1 (non-neuronopathic) Gaucher disease (GD), the most frequent lysosomal storage disease. **Methods**: The GD population included all patients with type 1 GD registered in the ICGG Gaucher Registry, who were diagnosed after 1991. Life expectancy was calculated according to the standard life table method by Palmore and Gardner, 1996 (method 1); and by using the assumption that risk of death prior to Gaucher diagnosis was the same as the reference population (method 2). The reference population (World Population Prospects, 2002, UN) was and approximately 40% of GD patients came from the U.S. The life expectancy of the reference population was similar to that for developed nations (as defined by the UN). The gender distribution in GD was similar to that of the general population. **Results**: The type 1 Gaucher population of 2,201 patients had 90 reported deaths. The average life expectancy of the Type 1 GD population was 66.6 years for method 1, and 67.3 years for method 2. The life expectancy of the reference population was 77.1 years. **Discussion and Conclusion**: The ICGG Gaucher Registry represents the largest dataset on GD patients worldwide. The current life expectancy at birth of people with type 1 GD is about 10 years less than the reference population, confirming that type 1 GD is not a benign disorder. Additional analyses on causes of death and a possible impact of enzyme replacement therapy on life expectancy are underway.

P1290. Recombinant expression of vitamin K-dependent coagulation-factors

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Vitamin K serves as a cofactor for the post-translational modification by gamma-carboxylation of several proteins with regulatory function, the group of Gla-proteins. Vitamin K-dependent proteins include the coagulation factors II, VII, IX, and X and proteins S, C and Z, illustrating the therapeutic importance of vitamin K-metabolism. Inhibitors of the coumarin-type like warfarin reduce coagulation activity by interfering with the vitamin K 2, 3-epoxide reductase enzyme complex (VKOR). Mutations in the VKORC1 (VKOR-component 1) -gene lead to combined deficiency of vitamin-K-dependent clotting factors type 2 (VKCFD2; OMIM: 607473) or warfarin resistance.

The production of vitamin K-dependent coagulation factors like factor IX which is being produced for treating Haemophilia B is challenging, because a significant amount of the recombinantly expressed protein is undercarboxylated. The supernatant of HEK-293-Cells which recombinantly express hFIX-cDNA driven by a CMV-promotor has an FIX-activity of 28% compared to normal blood-plasma. Interestingly when comparing the antigen concentration by Western blot-analysis the supernatant shows a much stronger signal than plasma. These data indicate that the recombinant FIX is insufficiently gamma-carboxylated. Since VKORC1 is the essential key-protein for gamma-carboxylation, we are trying to coexpress the coagulation factors II, VII, IX or X in combination with VKORC1 to improve carboxylation and thereby enhance the functionality of the expressed proteins.

P1291. Osteogenesis imperfecta in children, effects of therapy with pamidronate

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Osteogenesis imperfecta (OI) is a genetic disorder due to the decreased amount or abnormal structure of the collagen. Several forms with different symptoms affecting bones, teeth, sclera and growth are described.

Here we analyze symptoms in 9 children with OI (age 1 month-12 years at the diagnosis) and the response to therapy with bisphosphonates.

Multiple fractures were the major presenting sign except for 1 patient where the bowing of the lower legs was the presenting sign. The number of fractures before therapy was 2-22 (6.7±3 average). The most severe form of the disease appeared in a newborn and in a baby of 4 months, both with multiple fractures.

One patient where the OI was accompanied with empty sella showed only bowing of the legs without apparent fractures, the fact probably due to the GH therapy applied due to the GH deficiency. Two of the children had a familiar form of OI, mother being affected in both.

Biochemical studies and collagen studies confirmed OI type I in 3, OI type III in 4 and OI type II in 2 children.

Therapy with i.v. disodium pamidronate 1 mg/kg/month was conducted for 20±4.6 months. There were only 2 fractures during the total period of 95 months of therapy in all children. Two babies with the form II of the disease died due to severe infections. Seven are in a very good health with normal DEXA findings.

Treatment with bisphosphonates is successful in OI especially in forms I and III.

P1292. The individual approach in the Phenylketonuria treatment

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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 clinical and biochemical polymorphism. Low Phe diet usually used to treat PKU children can't insure amino acid balance, being not so efficient.

Method: 28 children with classical PKU were investigated for:

1. the level of amino acids in blood and urine through liquid chromatography based on amino acid analyzer Novo AAA339, Czech origin.

2. biochemical examination of blood and urine (especially Copper, Zinc, ceruloplasmin).

Based on the investigation results, these children received individual Low Phe diet and metabolical correction treatment, including drugs directly influencing the amino acids metabolism.

Results: of the investigations: disbalance in the transformation of Phe into Tyrozin; Phenylalaninuria; troubles in the metabolism of Methionine, Tryptophan, Histidine, Copper; functional troubles of the ornithine and Kori cycles. The application of drug metabolical correction, along with a Low Phe diet by indicating the smallest quantity of protein led to improvement of children's intellectual development. The IQ of PKU children who started such treatment from birth was > 85%.

Conclusions: PKU is a pathology of the whole metabolism and not a deregulation of one amino acid- Phenylalanine. The drug metabolical correction and Low Phe diet are prescribed individually according to the results of the examination of free amino acids in blood and urine. The screening test determines the fluctuation only of Phe in blood and can't be used in the individualized approach of the PKU treatment.

P1293. The Pompe Registry: Centralized data collection to outline the natural course of Pompe disease.

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Background: Pompe disease is a rare, progressive, and fatal muscular disease. The underlying pathology is a deficiency of acid alpha-glucosidase (GAA) that hydrolyzes lysosomal glycogen. Pompe disease is a heterogeneous disorder that varies with respect to age at onset and rate of disease progression.

Methods: To gain a better understanding of the natural course of Pompe disease, a global, observational Registry was developed to collect anonymous, longitudinal data on Pompe patients.

Preliminary Data Overview: As of January 11, 2006, 150 patients have been enrolled of which the majority (54.0%) is of Caucasian ethnicity. 18.0% of the reported patients have infantile-onset Pompe disease (IO: symptoms onset typically before the age of one year). The median age of IO diagnosis is 6.3 months. 54.0% of the reported patients have late-onset Pompe disease (LO: symptoms onset often after the age of one year). The median age of LO diagnosis is 31.4 years. The (median) range of time from symptoms onset to diagnosis is 6.7 months for IO patients and 7.5 years for LO patients. Out of 45 LO Pompe patients investigated for genotype, in 32 (71.1%) the IVS1-13T>G mutation was found.

Summary: The Pompe Registry attempts to increase the understanding of this rare disease and to potentially improve patient management. Preliminary data show that the (median) range of time from symptoms onset to diagnosis is similar to published literature, suggesting the need for greater disease awareness.

P1294. Bioaminergic deficits in Rett syndrome : from pathophysiology to therapeutic intervention.

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Rett syndrome is a severe X-linked neurological disorder, in which most patients carry a mutation in the gene encoding methyl-CpG binding protein 2 (MECP2). The clinical course of the disease consists of normal in utero and neonatal development followed by a period of regression showing signs of neurodevelopmental defects (arrest of brain development, loss of acquisitions such as speech and walk, apparition of behavioural troubles).

Twenty six percent of deaths in Rett girls occur with sudden respiratory arrhythmia.

We investigated breathing dysfunction in Rett syndrome using an animal model for the pathology deficient for the Mecp2 gene. We performed experiments on wild-type and Mecp2-deficient mice to understand the role of the Mecp2 gene in respiration and bioaminergic systems. We showed that adult mice deficient for the Mecp2 gene have erratic breathing with highly variable respiratory rhythm and frequent apneas, reduced norepinephrine content and a drastic reduction of tyrosine-hydroxylase expressing neurons in the medulla. We are currently investigating the stimulation of noradrenergic metabolism in the same animal model using specific noradrenaline reuptake inhibitors. Preliminary results show that we can improve both the respiratory rhythm of the mutant animals and increase significantly their lifespan. These results open new perspectives for the treatment of the respiratory phenotype of Rett syndrome children.

P1295. siRNA induced inhibition of *MRP1* expression and reversal of resistance in human promyelocytic cell line

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Multidrug resistance (MDR) is a complex phenomenon in which many different genes regulating drug transport, cellular repair, detoxification and drug metabolism will activate. Nevertheless, in most drug resistant cell lines and cancer patients, up-regulation of ABC-transporter genes such as multidrug associated protein gene (*MRP1*) could be at the basis of the resistance phenotype. We aimed to decrease the *MRP1* expression at mRNA level to modulate drug resistance phenotype in resistant HL-60 cell line. So we designed a small interfering RNA (siRNA) molecule against

MRP1 and used in HL-60 cell line in 0 to 72 hours time range. siRNA could specifically inhibit gene expression at 40 to 44 hours up to 90% at the mRNA level when *MRP1* mRNA quantified by real time RT-PCR.. siRNA treated cells demonstrated four-fold reduction of methotrexate compared with untreated cells. The data indicate that this approach may be applicable to cancer patients to reverse resistant tumors with a *MRP1* dependent MDR phenotype back to a drug-sensitive one.

EMPAG Plenary Lecures

EPL01. Informed decision making in the context of prenatal screening

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This study aimed to construct a measure of informed decision making that includes knowledge, deliberation, and value-consistency, and to assess the level of informed decision making about prenatal screening, and differences between test acceptors and test decliners.

Women attending one of 44 midwifery and gynaecology practices were asked to fill out postal questionnaires before and after the prenatal screening offer. The principal outcome was the level of informed decision making. For this purpose, knowledge about prenatal screening, deliberation about the pros and cons of the alternatives, test uptake, and attitude towards having a prenatal screening test were measured.

84% of the participants were sufficiently knowledgeable about prenatal screening, 75% of the decisions were deliberate, and 82% were value-consistent. 51% of the participants made an informed decision. Test acceptors made less informed decisions as compared to test decliners. This difference was mainly caused by the lower rate of deliberation in this group.

It appears from this study that prenatal screening decisions are often not informed decisions. This is inconsistent with the main objective of offering screening, which is to enable people to make informed decisions. Decision makers should be encouraged during the counselling to deliberate about the various alternatives.

EPL02. Decision-making for invasive prenatal testing: the role of ambivalence

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A central goal of prenatal genetic counseling is for clients to make optimal decisions about invasive testing. An optimal decision can be operationalized as an informed choice; understanding relevant information, choosing a course of action consistent with one's attitudes, and resulting in minimal decisional conflict or regret. Dual processing theories offer a robust framework for studies into prenatal testing decision-making by addressing two types of information processing, rational and experiential. Experiential processing has been largely ignored in studies of prenatal decision-making. We hypothesize that since decisions about prenatal testing involve new and unfamiliar choices with significant potential gains and losses, experiential processing may explain a greater degree of the variance in predictors of decisions. Attitudes toward testing have been shown to be strong predictors of decisions. When attitudes are in conflict, ambivalence toward prenatal testing exists and unveils an opportunity for interventions aimed at prioritizing conflicting values or beliefs. We demonstrate ambivalence in a qualitative study of 36 women facing decisions about prenatal testing. Quantitative data from a larger cohort of women ascertained from five US clinics also reveals ambivalence. Given the prevalence of ambivalence felt by women facing prenatal decisions, interventions aimed at engaging experiential processing can be tested for their promise in making optimal decisions.

EPL03. Does a decision aid for prenatal testing of fetal abnormalities improve women's informed decision-making? Results from a cluster randomised trial

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Women's informed prenatal testing decision making has never been more important with the development and increasing utilisation of first trimester tests.

A cluster randomised controlled trial of 55 General Practitioners (GPs) was conducted to explore whether a decision aid, when compared to a pamphlet, assists women's prenatal testing decision making. GPs were randomised to one of two arms: providing women with a decision aid or a pamphlet. Primary outcomes, informed choice and decisional conflict, were measured at 14 weeks gestation using questionnaire data.

Both arm of the trial had a response rate of 77% (N= 337). Women had a mean age of 31 years, 37% were primigravid and 87% born in Australia. 68% had a 'good' level of knowledge in the decision aid group compared to 48% in the pamphlet group (Adjusted OR 2.38 95% CI 1.23 to 4.58). The odds of women making an informed choice was almost twice as large in the decision aid group compared to the pamphlet group and after adjusting for confounders this approached statistical significance (95% CI 0.99 to 3.71, p = 0.06). Mean decisional conflict scores were very low in both groups (decision aid 1.70; pamphlet 1.64): not a statistically significant difference. There were no significant differences in measures of depression, anxiety or attitudes to the pregnancy/fetus.

Use of a tailored made information resource can produce an improvement in women's knowledge about the complexities of prenatal genetic testing. This resource can potentially play an important role in improving women's informed decision making.

EPL04. Psychological consequences of receiving a positive screening outcome

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The aim of this study was to gain more insight into how women look back upon their pregnancy when they had received a (false) positive screening outcome and how they feel about having been offered a prenatal screening test. In the Netherlands it was not allowed to offer prenatal screening tests, a special license was obtained to perform this study.

Data were used of women who received a false-positive screening result in the context of a larger study in which women were offered a prenatal screening test and filled in several questionnaires during and after pregnancy. At random, 13 of the 25 women who received a positive screening outcome, were selected to be interviewed, a year after delivery. Data of both questionnaires and interviews were used.

Before they had the screening test done, hardly any of the women had thought about what they would do when the screening outcome would be bad. They all assumed that the outcome would be that the child was fine. In the period between receiving the increased test result and the negative diagnostic test outcome, women took more distance from their pregnancies. After they received the negative (good) diagnostic test outcome, most women were relieved. However, in some women doubts remained, because they still attached meaning to the outcome of the screening test which had shown something was wrong.

Despite the negative experience, most women were positive about the fact that they had been offered a prenatal screening test.

EPL05. Confidentiality versus duty to inform - An empirical study on attitudes towards the handling of genetic information.

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To our knowledge there are heretofore no empirical studies looking at whether potential relatives want to be informed about the existence of hereditary conditions within their family, and under which conditions they want healthcare providers to breach confidentiality in order to be informed. The purpose of the following study was to investigate attitudes towards these issues among the general public. It was hypothesized that willingness to be informed would be influenced by characteristics of the disease and the individual.

Three survey studies where undertaken in Norway and Sweden. Surveys were administered to a Norwegian random sample (N=2400) to a Swedish random sample (N=1200), and to a Norwegian student sample (n=607). Participants were asked to imagine that they had a

relative with an unspecified hereditary disease. Eight different disease scenarios were constructed, systematically varying three disease characteristics: fatality, penetrance and availability of treatment. Individual characteristics were measured by the following scales: General-Self-efficacy, Attitude-toward-Uncertainty, Penn-State-Worry-Questionnaire.
Results show that a majority of participants wishes to be informed about the existence of a hereditary disease within their family. Significantly more participants want to be informed for treatable compared to non-treatable diseases. Neither fatality nor penetrance did by themselves influence wishes to be informed. However the number of participants accepting breaches of confidentiality is greatest for the disease that is treatable, highly penetrant, and fatal. Regarding individual characteristics results show that wishes to be informed and acceptance of breaches of confidentiality are predicted by uncertainty avoidance and age, but not by self-efficacy or worry.

EPL06. Open family communication is positively associated with the well-being of individuals opting for genetic susceptibility testing for BRCA1/2 or HNPCC

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Objective of the study was to evaluate family communication, family functioning, differentiation to parents and support from relatives as predictors of psychological distress and adverse consequences for family relationships in individuals undergoing genetic testing for a cancer susceptibility.
The family system characteristics were assessed in 271 applicants for genetic testing of a known familial mutation in BRCA1/2 or a HNPCC related gene before genetic test result disclosure. Hereditary cancer distress and worry were assessed before, one week after, and six months after result disclosure. The prevalence and nature of adverse consequences on family relationships were assessed six months after result disclosure. Regression analysis procedure backward elimination was used to identify the predictive qualities of family system characteristics.
Hereditary cancer distress over the study period was associated with inhibited communication about hereditary cancer in the nuclear family and the family of origin. A minority reported unwanted changes in family relationships (19%), problematic situations in the family (13%) or family conflicts (4%). Adverse effects consisted of feelings of guilt towards children and carrier siblings, imposed secrecy and communication problems. Predictors of adverse consequences on family relationships were inhibited communication about hereditary cancer with relatives, and disengaged-rigid or enmeshed-chaotic nuclear family functioning. Our data support the need to stimulate open family communication in genetic counseling, because absence of open communication is associated with genetic testing related distress and familial adverse effects.

EPL07. Non-maternity: Issues for the family and the genetic counsellor

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Non-maternity is a rare occurrence in clinical genetics, compared to the not uncommon experience of non-paternity. Most people are confident about who their mother is or isn't, but for women, concealing the true biological maternity of the child they raise, is fraught with pitfalls.
Three cases of reported non-maternity are explored and the complex issues raised examined. In one family the 'child' had lived their life under the mistaken belief that the woman who brought them up was their birth mother. Subsequent genetic investigations led to a disclosure of the truth by the 'mother' with traumatic results for the family .In other families the truth is known within the family and not disclosed to those outside unless there is a need to do so.
Adoption laws have changed and the process of legally raising another's biological child is now more transparent; framed by legal processes, rights and guidelines. This encourages more open communication within the family and with the outside world. Contraception and the availability of social abortion have made hidden pregnancies less common. However egg donation raises differing issues for families at risk of genetic conditions. Recent changes in the law in the UK, allow children born using eggs donated after April 2005 to trace their biological mother when they reach 18 years. As geneticists and genetic counsellors we need to be mindful that for some families the taking of a family history and the offering of genetic testing may have far wider implications than was predicted.

EPL08. An exploration of the attitudes and educational needs of people considering cystic fibrosis carrier screening in Victoria, Australia.

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20 children are born with cystic fibrosis each year in Victoria. In most cases there is no family history of CF, hence the importance of a CF carrier screening program. Evidence from national and international groups demonstrates that both providers and consumers have positive attitudes regarding genetic carrier screening for cystic fibrosis.
This study explores the attitudes of stakeholders to a cystic fibrosis genetic carrier screening program in Victoria, Australia.
Non-random, purposive sampling was used to recruit participants for focus groups of key stakeholders: preconception individuals, pregnant women, health professionals and cystic fibrosis experts. The discussions were audio taped and transcribed and thematic analysis was facilitated by the qualitative data management software, "nVivo".
Participants expressed mixed attitudes towards genetic carrier screening for cystic fibrosis. Consumers' attitudes were influenced by an awareness of family history, the opinions of health professionals and their partners and, their own values and beliefs. Providers' attitudes were influenced by time constraints in practice and concerns for the psychosocial outcomes of patients.
Participants discussed the different settings in which screening may be offered and were most strongly in support of preconception screening however, the practical barriers to offering screening at this time were recognised. Participants proposed that screening be offered when people are "ready" to be screened, ie offering screening at different life stages.
Whilst attitudes to a genetic carrier screening program for CF are mixed, community interest is growing. Consultation with stakeholders is vital to inform the development and implementation of successful genetic carrier screening programs.

EPL09. Comparison of the psychosocial support offered to breast cancer patients and healthy family members.

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Psychosocial support for genetic testing in the Netherlands is offered by social workers in medical genetics departments in the university hospitals. Initially, this support in relation to DNA testing in hereditary breast cancer focused on healthy family members at risk, but it soon became clear that breast cancer patients are also a vulnerable group. They, too, are confronted with the consequences of the genetic diagnosis: knowing the risks of a second breast cancer, of ovarian cancer and for their children and close relatives. It is now recognised that a diagnosis of hereditary breast and ovarian cancer (HBOC) has many consequences, for both patients and healthy family members.
We present an overview of the need for professional psychosocial support in HBOC patients and healthy family members. We collected data from 125 counselees (40 breast cancer patients and 85 applicants for predictive testing) on their need for professional psychosocial support, the treatment plans and the types of interventions we used. We compared data from the literature with the results from this study. Emotional support in coming to terms with the diagnosis, decision counselling, and coaching in family communication are the most frequent types of help, while task-centred therapy, family-focused interventions and psycho-education are examples of common interventions.

The results show that the distinction between predictive and diagnostic testing is artificial and is not an indicator of the individual's need for professional support. However, there are similarities and differences in the issues presented by counselees and the interventions offered by the social worker.

EPL10. Interdisciplinary genetic counselling for families at risk of HNPCC: Impact on psychosocial outcome in affected and unaffected participants

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Purpose: Genetic counselling and testing is offered to families with suspected Hereditary Non-polyposis Colorectal Cancer (HNPCC) since 1999, funded by the German cancer AID. In this study, the impact of a comprehensive counselling protocol is explored, with regard to counsellees' psychological distress, beliefs specific to HNPCC and family communication. Since little information is available about affected cancer patients' psychosocial status with regard to HNPCC, the response to genetic counselling of affected and unaffected participants is compared.

Methods: Multidisciplinary counselling consists of three consecutive sessions, provided by a geneticist, a visceral surgeon and a psychotherapist. Risk assessment based on clinical criteria (i.e.Amsterdam/Bethesda) is conveyed to participants. Distress and beliefs specific to HNPCC were assessed using standardised as well as investigator-derived measures, completed before and 8 weeks after counselling. The final sample size comprises 379 participants (141 affected, 238 unaffected).

Results: After counselling, a significant reduction in general anxiety (HADS) and distress specific to HNPCC (IES, DHD) was demonstrated, in both affected and unaffected subjects. Distress declined regardless of what clinical risk they were assigned. Their perceptions of cancer-related threat declined while confidence in effective surveillance increased. Enhanced family communication with regard to HNPCC was reported by one third of the counsellees. Affected patients' distress exceeded that of unaffected subjects at both time points.

Conclusion: The results are suggestive of a beneficial effect of comprehensive genetic counselling, even when high risk information is conveyed. Clinically relevant distress in a substantial minority of affected patients is indicating the need for appropriate counselling.

EPL11. Internet use among cancer patients attending breast cancer genetic clinics

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INTRODUCTION The rates of Health-related Internet use by cancer patients vary between 8% and 50% in the literature.

Objective: To present baseline data on the access to Internet by French breast cancer patients attending at genetic clinics and to examine factors affecting these patients' rates of Internet health-related use.

METHODS This multi-centre (N=11) survey included affected women attending at breast cancer genetic clinics and who were offered BRCA1/2 genetic testing. Self-administered questionnaires collected sociodemographic details and information about Internet access and use.

RESULTS Among the 560 respondents (response rate=87%), 272 (48.6%) had access to Internet and 136 (24.3%) consulted Internet to obtain health information.

In those with access to Internet, health related Internet use decreased with age but increased with the educational level, among those with a health-related occupation and among those with a low probability (<25%) of a BRCA1/2 mutation being identified (**Table**).

The opinions about the visited websites were positive (24%), negative (32%) or both (11%); 33% had no specific opinion. Patients reported difficulty to understand detailed medical website information and would have preferred to be given a specific address to consult.

DISCUSSION Health-related use of Internet by French cancer patients seems to be one of the lowest reported so far in cancer patients. Almost 1 out of every 3 women were dissatisfied of this source of information.

Predictors of health-related Internet use in women with access to Internet (N=272)

	Health-related Internet use among those with access							
	Yes (n=136)		No (n=135)					
	n	(%)	n	(%)	Chi² test *P* Value	adjusted Odds Ratio*	95% Confidence Interval	
Age					<0.001			
> 60	4	(19)	17	(81)		1		
41-60	85	(47)	95	(53)		**3.9**	1.2	12.8
<=40	47	(67)	23	(33)		**7.8**	2.2	27.8
Education					<0.001			
Lower or equal to high school	48	(46)	56	(54)		1		
College (1-3 years)	33	(38)	54	(62)		0.7	0.3	1.2
College (>=4 years)	54	(69)	24	(31)		**2.1**	1.1	4.1
Health-related occupation					0.002			
No	97	(46)	116	(54)		1		
Yes	37	(70)	16	(30)		**3.0**	1.5	6.0
Probability of a BRCA1/2 mutation being identified					0.027			
>=25%	65	(44)	82	(56)		1		
<25%	71	(58)	52	(42)		**2.2**	1.3	3.8

* Logistic regression analysis predicting health-related Internet use

EPL12. Evaluation of clinical genetics services - a qualitative study identifying outcome measures

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Outcome measurement in clinical genetics has been a long-standing challenge. To date, much research investigating outcomes has focussed on the benefits of genetic counselling. The outcome attributes used were commonly developed by providers of the service or adapted from measures used in other areas of healthcare. However, unlike other areas of medicine the 'patients' in clinical genetics are usually healthy; often no specific pharmacological or surgical treatment is offered. There is an argument that services should be evaluated on the basis of how well they alleviate the effects of disease, from a patient perspective. The first step, therefore, is to identify what those effects are. This presentation will describe findings from focus groups and interviews conducted with clinicians, patients and patient representatives. The approach differs from previous outcome research in clinical genetics, by using combined methods to identify firstly what the effects of genetic diseases on individuals and families are, and then to assess what genetics services can do to modify these effects. A series of social and emotional effects of genetics diseases were identified, and these will be described along with interventions identified that can help alleviate

some of these effects. The presentation will include some discussion of how and why findings differed between focus groups and individual interviews. The "outcome attributes" identified in this research will be used in the evaluation of existing outcome measures used in clinical genetics, and may be used in the longer term to develop robust measures of outcome for clinical genetics services.

EPL13. A ten year follow-up study of predictive testing for breast / ovarian cancer in two generations of five large *BRCA1*-linked families

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We report the uptake and long-term outcomes of offering *BRCA1* presymptomatic genetic testing to 100 individuals in 2 generations of 5 large *BRCA1* families for the first time. Initial testing was offered with linkage and then subsequently with mutation analysis. Uptake was significantly higher in the first generation, who were directly offered testing, and much higher in females; 31/42 (74%) of unaffected women in the first generation proceeded to testing compared to 13/31 (42%) of men. This decreased to 7/16 (44%) of women in the second generation and (0/11) males (p=0.0004). GHQ scores decreased in the year following the mutation result in all groups, but mean scores rose at the 10-year point, although this did not appear to be associated with cancer worry. All responders (76%) to a 10 year questionnaire indicated that they were pleased they had undertaken the predictive test, and all but 2 were highly satisfied or satisfied with the whole process. Benefits of testing were reported by both mutation carriers and non-carriers. These results suggest that long-term outcomes of offering genetic testing are very acceptable.

EPL14. Cognitive and Behavioural adjustments two years after an inconclusive genetic test result in a cohort of HBOC affected women

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Little is known about how women who receive an inconclusive result from BRCA1/2 testing interpret their result. No long term prospective study is available on cognitive and behavioral adjustment they develop to cope with this uncertainty .

Our objective was to explore affected women's cognitive and behavioral adjustments to an inconclusive BRCA1/2 test result and to what extent these adjustments were linked.

This study was carried out on 83 women with personal and familial breast/ovarian history of cancer, who received inconclusive result to genetic testing two years before. Self-administered questionnaires were prospectively collected. Here we present a qualitative analysis of open written commentaries obtained on risk perception and diffusion of information and when relevant, of corresponding closed questions.

61.4% women made commentaries on genetic predisposition. There did not differ for socio-demographic characteristics from those who did not. We observed three types of reactions to inconclusive result : 11 women coped with the uncertainty of the result , 9 women turned out the meaning of the "negative result" into a certainty and considered they were not at a higher risk anymore, last group (6 women), continued to be convinced to be at-risk, given the personal history of cancer. In every group, behaviours they declared to adopt towards diffusion of information to family and preventive strategies will be presented.

Our findings show the precautions that practitioner must take to ensure that women with inconclusive results understand that their family remains, most of the time, at a high risk of developing breast/ovarian cancer.

EPL15. Do clinical characteristics affect the impact of an uninformative DNA-test result? Course of worry and distress of test applicants for breast cancer.

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Introduction A DNA-mutation test result for breast cancer is usually uninformative given that there has been no mutation detected within family members previously. However, few data are available on the psychological impact of this common type of result. Moreover, the clinical heterogeneity within this group has not yet been considered. The current study provides prospective data about the course of cancer-specific worry and distress for different groups of test applicants.

Methods All DNA-test applicants (n=238) completed three questionnaires: before, and respectively one and seven months after disclosure of a DNA-mutation test. With repeated measures analysis of variance, differences were assessed between BRCA1/2-positive women (n=42), BRCA1/2 true-negative women (n=43), and women with an uninformative result (n=153).

Results On group level women with an uninformative result seemed to be reassured after disclosure (P<.001), but to a lesser extent than those women who received a true-negative result. However, not all women with an uninformative result reacted similarly: Higher levels of worry and distress could be explained by relatively straightforward clinical variables, namely a personal history of cancer ($P\leq$.001) and a higher pedigree-based risk ($P\leq$.005). Furthermore, these clinical variables determined whether these women were either comparable to women who received a true-negative result or to BRCA mutation carriers.

Conclusion Women with an inconclusive result form a heterogeneous group of test applicants. The subpopulation of those with both a personal history of cancer and a relatively high pedigree-based risk expressed the highest levels of worry seven months after DNA testing.

EPL16. The impact of predictive genetic testing for hereditary nonpolyposis colorectal cancer (HNPCC) - Three years after testing

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This Australian longitudinal multi-centre study assessed the impact of predictive genetic testing for HNPCC on psychological wellbeing and preventive behaviours. Questionnaires were sent prior to (baseline), and two weeks, four months, one and three years after receiving genetic test results. Psychological measures were included at each time and preventive behaviours assessed at baseline, one and three years.

At three years, 19 carriers and 54 non-carriers (n=73) responded - 64% of baseline. Of non-responders, 2 had died and 5 developed cancer. Non-responders had significantly higher mean cancer-specific distress than responders at baseline but mean depression and general anxiety scores were not significantly different. Similar proportions of each group were carriers.

Mean depression and generalised anxiety scores did not differ between carriers and non-carriers, and at 3-years, were similar to baseline. Adjusting for age, gender and baseline score (ANCOVA), carriers had higher mean cancer-specific distress scores than non-carriers at one-year (p=0.021) and three-years (p=0.088). Carriers showed an increase in mean score of cancer-specific distress at 2-weeks with a decrease by 4- and 12-months and a slight increase again at 3-years (not significant). Non-carriers showed a sustained decrease after testing.

All carriers and 7% of non-carriers had colonoscopy and 69% of 13

female carriers had gynaecological screening in the previous two years. Prophylactic surgery was rare.
This is the first report of long-term data after predictive testing, on carriers and non-carriers of HNPCC mutations. These results indicate appropriate screening, improved psychological measures for non-carriers and no evidence of undue psychological distress in carriers.

EPL17. Reproductive decisions in asymptomatic carriers of the Huntington-mutation.

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When genetic testing became available for HD, new possibilities regarding reproduction emerged for couples having an increased risk of HD in the fetus. The aim of this study is to describe reproductive decision making in asymptomatic carriers of the HD-mutation.
Methods: Psychological counselling has been systematically offered at 1 week, 1 month, 1 year and 5 years after predictive testing. Moreover, several tested persons had additional follow-up sessions. During all follow-up sessions, data on reproductive decisions were collected by means of (clinical) interviews. The follow-up period in this study was 1 to 16 years.
Results: In the period 1987-2004, 245 individuals received a test result in Leuven. Eighty-nine of them were carriers and 7 received an equivocal result. For 46 carriers and 2 persons with an equivocal result, reproductive decisions was one of the motives for predictive testing. During the follow-up period in this study, nearly half of the carriers had children born after either prenatal diagnosis (PD), either preimplantation genetic diagnosis (PGD). About 1 in 3 carriers had decided to have no own children after the test. Further details on reproductive decisions will be presented. We also present some qualitative data on perceived advantages and disadvantages of predictive testing, PD and PGD; decision conflicts; moral pressure; technological imperative, ….
The results show that reproductive decision making is a complex process with rational and emotional aspects, as well as irrational and unconscious elements. Implications for psychological counselling will also be formulated.

EPL18. The experiences of men and their partners during pregnancies at genetic risk

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Men rarely feature in literature on genetic risk and its impact during pregnancy. Drawing on data from a prospective study of 49 pregnancies at high genetic risk, we present qualitative data from 10 individuals (5 women and their 5 male partners) whose pregnancy was at risk from a known parental translocation.
Prospective data was collected at 3 points: by interview soon after a pregnancy was confirmed, 6 months later by questionnaire and by interview after a further 6 months.
This unique data set allowed the impact of genetic risk and testing during pregnancy to be examined from gender and time perspectives. Interviews were taped and transcribed, and analyzed using qualitative techniques.
Key themes identified include timing of emotional attachment to pregnancies, guilt and blame, and pregnancy related support. For example, attachment developed later for the men than the women, with some men reporting attachment only after a child was born. Draper's concepts of 'biological exclusion' and 'blurred boundaries' inform our discussion of this. Issues of guilt and blame were raised independently by the three male carriers of a translocation, but not by the two men whose partner was the carrier. While some men illustrated their own need for support, all adhered to normative roles of the 'provider of support' during a pregnancy. These findings from a small sample cannot be generalized, but they add insight into the impact of pregnancies at genetic risk on men and their partners, and give support to the need for further research in this area.

EPL19. Reproductive decision-making in women with Marfan syndrome

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Marfan syndrome is a dominantly inherited connective tissue disorder. The cardiovascular, musculoskeletal and ocular systems are commonly affected. In pregnancy, women with Marfan syndrome face an increased risk of cardiac complications - such as aortic dilation/dissection - in addition to the 50% chance of having an affected child. Semi-structured interviews were used to explore the process of reproductive decision-making in seven women with a clinical diagnosis of Marfan syndrome. The women tended to minimise the impact of the condition on themselves; even so, their perceptions of the severity of Marfan syndrome were influenced by family members' experiences which contributed to a belief that the condition would worsen over time. Having Marfan syndrome had not stopped any of the women having children, although in most cases conception of the first child had either been unplanned or had occurred when the women were in a state of denial or avoidance of the condition. The birth of their first child led to women moving from a state of denial or avoidance of the condition to one of taking control, by becoming informed and participating in medical surveillance. A number of factors were influential when the women were considering whether to have further children: their concern about their future health, mortality and morbidity; their feelings about passing on Marfan syndrome to their children; their current health; and, the concerns and/or opinions of other family members. Many of the women believed that having Marfan syndrome had or would limit the size of their family.

EPL20. Targeted testing in prenatal diagnosis: the best way to deal with problematic findings?

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Besides being a rapid way of prenatal testing, targeted testing also provides the option to exclude testing results which are considered too problematic to counsel. To assess if this new method of testing is the obvious way to deal with problematic findings, these findings were systematically examined.
Providers from several disciplines were asked, individually and in focus group discussion, about their experiences with various testing results in general, and problematic results in particular. Clients were asked about their expectations and experiences regarding the same matter. Providers' and clients' experiences were compared and analysed to search for themes related to problematic testing results.
Problematic results arise because providers, wishing to avoid underreporting, also report results of mild or unknown clinical significance. In a context where only two choices, i.e. continuing or terminating the pregnancy, are available, these results may lead to big dilemmas. Because in the end, the decision about pregnancy is considered the clients' responsibility, clients are more troubled by problematic results than providers.
The burden for clients would be diminished in a targeted testing scenario where providers decide which problematic results would be excluded, more than in a scenario where clients would decide about this. From the perspective of a fair distribution of responsibilities between providers and clients, targeted testing would be the obvious way to deal with problematic findings in prenatal diagnosis. Consequently, the providers would need to answer the morally laden question of which testing results should be excluded from prenatal diagnosis.

EPL21. Presymptomatic testing in Myotonic Dystrophy type I and Facioscapulohumeral Muscular Dystrophy : 6-year experience

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We report a 6-year experience of presymptomatic testing in 131 candidates for Myotonic Dystrophy type I (DM1) and 50 candidates for Facio-Scapulo-Humeral muscular dystrophy (FSH) with a protocol characterized by multistep procedure in a multidisciplinary team. We compare characteristics and outcomes of theses subjects with previous reported data about Huntington's disease and autosomal dominant cerebellar ataxias. This is the first detailed report and the largest series reported concerning candidates for presymptomatic testing in DM1 and FSH, whatever their attitude towards the testing procedure.

The characteristics of applicants were similar in DM1 and FSH, revealing a predominance of women, a high rate of favorable results (64% for DM1 and 62% for FSH), and family planning as the most frequent reason for seeking presymptomatic testing (31% for DM1 and 28% for FSH). For FSH, we observed a low rate of completing the presymptomatic testing program (58%). For DM1, the rate of completing the presymptomatic testing program was higher than in others previously studied diseases (87%). Despite equivalent socioeconomic characteristic DM1 cannot be compared to others diseases in term of comportment face to the presymptomatic testing protocol. Problems of congenital form, cardiac risk and anticipation lead to a feeling of emergency and an incentive to do PT with familial and medical pressure over at risk subjects. These observations enhance the importance of counselling in multistep and multidisciplinary teams to clarify stakes and motivations for each subject and to anticipate consequences of an unfavourable result.

EPL22. Genetic testing for familial cardiovascular conditions in minors; their perception of the implications of their carrier status.

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Predictive genetic testing for familial cardiovascular conditions such as Familial Hypercholesterolemia (FH), the Long QT syndrome (LQTS) and Hypertrophic Cardiomyopathy (HCM) has become possible in families in which the causative mutation has been identified. The most devastating possible consequence of these diseases is sudden death, sometimes in minors. Carriers can reduce their risk by the use of medication and adaptation of life style. The latter may involve diet restrictions or avoidance of triggering situations such as competitive sports, stress or loud noises.

In the case of minors, the decision to undergo genetic testing lies with the parents. However, testing of minors is a point of (ethical) debate. An issue in this debate is how knowledge of their carrier status might benefit or harm minors. So far, children's experiences in this regard are unknown. Therefore, a qualitative study was initiated to explore the cognitive, behavioral and emotional impact of carriership on minors (aged between 8 and 18 years).

We interviewed 35 minors with a positive carrier status from 30 families. Semi-structured interviews were conducted at the families' homes. Minors were interviewed separately from their parents. All interviews were transcribed verbatim. Two researchers labeled these transcriptions independently. Discrepancies between coders were negotiated until consensus was reached.

This presentation will focus on minors' perception of the identity, causes, consequences, controllability and timeline of their condition and how this perception might relate to medication use, adaptations in lifestyle and worries. Clear differences in perceived controllability emerged and these appear to be associated with worries.

EPL23. Emotional experiences and representations associated with the psychological development of pre-symptomatic individuals with Familial Amyloid Polyneuropathy, type I (FAP ATTRV30M) - Portuguese, Andrade

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Individuals who live in a psychological environment threatened by the disease/loss/death of a parent organize their reactions to psychic suffering and emotional expression, according to their life experiences during childhood. Our aim was to understand the experiences and representations associated with some aspects of psychological development of individuals who come for presymptomatic testing of FAP-ATTRV30M.

We interviewed 91 individuals at risk (44 men, 47 women), aged 18-66yrs (mean 29). Items analysed included: own evaluation of childhood and memories, diseases, dreams and graphic expression; psychomotor development; breast-feeding; separations/changes, relationships.

Most (76%) claimed to have had "normal/good" childhoods; few (11%) recalled "bad/difficult" memories of that period; 57% referred family (mostly parents) when mentioning childhood memories. Moreover, they also mentioned the disease/death of a parent; 24% described symptoms of somatic expression during childhood. The majority did not remember their dreams (55% for past, 52% for present dreams), or remembered only dreams with a threatening content; 76% acknowledged having been breast-fed; almost all initiated a stable relationship in late adolescence, and usually got married quite soon (98% had a stable relationship, 64% referred having had few relationships).

FAP possibly leads these families to develop strong cohesion mechanisms that give them the perception of proximity/support/safety, which generates the idea of a "good/normal" childhood. They do not explicitly express psychological suffering. Repression of oniric expression and a tendency to somatize suggest a blockade of symbolic activity. The probability of these individuals later suffering from FAP may lead them to anticipate relationships, marriage and other life aspects.

EPL24. Genetic risk and gender: Current understanding and clinical implications

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There is increasing evidence that men and women experience genetic risk and engage with predictive genetic testing differentially. Current research literature suggests that at-risk women are significantly more likely than their male counterparts to undertake predictive testing with regard to inherited neurological conditions such as Huntington's disease, as well as inherited cancers and other genetic conditions. What factors might account for this and what are the implications of this phenomenon for clinical practice? Drawing on a range of theoretical literature and social science and clinical genetics research, this paper examines the concept of gender and how it may influence the perception and experience of genetic risk and engagement with genetic testing technology. Gender-based analysis of selected data from two Australian-based studies will be presented to further our understanding of this issue: firstly, from the Genetic Discrimination Project in Australia which investigated the experiences and perceptions of 904 asymptomatic individuals who had undertaken predictive genetic testing for inherited neurological disorders (n=332), familial cancers (n=481), haemochromatosis (n=45) and other genetic conditions (n=46); and secondly, from an in-depth interview study that investigated the post-genetic-test experiences of at-risk men and women who undertook predictive testing for a range of genetic conditions. Gender-based analyses of both quantitative and qualitative data from these studies will be presented and discussed in light of current literature and research. The potential implications of these findings within the clinical genetics context will be discussed.

EPL25. Experiences and attitudes of Turkish Cypriots in Cyprus about testing for Thalassaemia carrier status

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Currently, some groups undergo or are offered carrier testing for recessive conditions, e.g. beta thalassaemia, that are common in their populations/ethnic groups. This can enable couples to make informed reproductive choices, and may also reduce the number of affected individuals in subsequent generations. Genetic testing to identify beta thalassaemia carriers in the Turkish Cypriot community in Cyprus has been carried out for the past 25 years. It is mandatory for couples to be tested before a marriage certificate can be issued, though the results of the test have no bearing on whether the certificate is issued or not. No previous research has investigated individuals' attitudes towards and views about compulsory carrier testing in populations where this happen.

Semi structured interviews with 19 Turkish Cypriots, 11 women and eight men, who had undergone mandatory testing were carried out to gain an insight into their views about this. Ten of the interviewees are carriers and nine are non-carriers. There were six couples, two of whom were carrier couples. A number of themes have emerged from this. The interviewees identified that more education about the condition itself and about testing would be helpful. They felt it would have been important to have had more counselling before and after they were tested. Also, even though carrier testing was seen as important by most interviewees, some felt it had been imposed on them. Finally, a number of interviewees felt carrier testing should have been done earlier, and not around the time of their wedding.

EPL26. Invasive procedure uptake rate among women of advanced maternal age in Johannesburg - cultural aspects

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A recent review of the amniocentesis uptake rate in a population of women in Johannesburg, South Africa has revealed a low uptake rate compared to other studies. The division of Human Genetics of the University of the Witwatersrand and the National Health Laboratory service provides genetic counselling and invasive testing to women of advanced maternal age (AMA) (>35 years). A retrospective patient record audit was performed over the period January 2003 to December 2004. A total of 696 women were seen during this period, 524 women were of advanced maternal age only (AMA), and 172 women were of advanced maternal age and another indication (AMA P). The population group distribution were; 577 (83%) black, 53 (8%) coloured, 28 (4%) white, 14 (2%) indian, with 23 (3%) not recorded. A total of 208 (30%) women chose an invasive test after genetic counselling (8 had chorionic villus sampling performed). The uptake rates were; 27% in the black group, 33% in the coloured group, 14% in the indian group and 42% in the white group. The reasons women provided for deciding against invasive testing were recorded. The three reasons stated most often by the women in the black population group were; will not terminate an affected baby, had to discuss with partner/family; and afraid of miscarriage risk. The meaning of these statements within the context of the population and cultural groups in South Africa will be discussed as well as the impact on the current genetic counselling service offered to patients.

EPL27. Understandings of Down's syndrome: a Q methodological investigation.

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Despite the development of prenatal tests for a growing number of conditions, Down's syndrome has been, and continues to be, a central focus of prenatal testing technology. With the growing concern for supporting informed prenatal testing decisions it is surprising that there has been little examination of how attitudes towards this condition influence these decisions. This study used Q-methodology to identify how different understandings of Down's syndrome might relate to attitudes towards the use of prenatal testing and termination for the condition. Seventy-six people were selected as being likely to represent a diverse range of views about Down's syndrome, approximately half of whom had some known experience or expertise related either to the condition or to prenatal testing. The participants Q sorted 50 propositions about Down's syndrome selected to reflect different views about the condition in terms of its impact on the affected person, on families with an affected child, and on society. Six statistically independent accounts of the condition reflecting a range of attitudes towards, and experiences of, people with Down's syndrome were extracted using Principal Components Analysis. The study demonstrated that people hold complex and sometimes seemingly contradictory views about Down's syndrome, and that these are likely to influence their prenatal testing decisions. The current antenatal setting provides little opportunity for people to discuss and explore their views on disability. It is argued that this may affect the ability of some individuals to make decisions that are informed by their own views and values.

EPL28. The influence of traumatic experience of the cardiac arrest and sudden death on perception genetic information and predictive testing means

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Inherited cardiac channelopathies are heterogeneous group of diseases with a high risk of life-threatening episodes and cardiac sudden death (SCD).

Aim: To analyze the factors determining perception of the genetic counseling and needs of the DNA diagnostics in patients with inherited arrhythmias.

Methods: We have been interviewed 40 adult patients and their relatives from 25 unrelated families with Long QT syndrome and Brugada syndrome. For all individuals genetic counseling were recommended because of clinical diagnosis of primary channelopathies or of relations to affected patients. All individuals were asked about their clinical and family histories, pedigrees, causes of the death in relatives, requests for genetic testing and attitude to predictive genetic testing.

Results: We have been subdivided tested persons into two groups: (1) patients survived through cardiac arrest or had SCD victims among near relatives and (2) patients had mild clinical course of the disease and hadn't dramatic experience of SCD in their families. About 80% patients from group (1) noted molecular genetic testing as important point of examination and strongly intended to use the results in the next family planning for themselves and for their issue. In the group (2) the individuals pointed to lower interest in DNA testing and prenatal diagnostics.

Conclusion: Personal and family experience of SCD is the most important factor influencing on decision-making in the inherited arrhythmias patients. Possibility to possess healthy issue is the integral part of the good quality of life for patients with high risk of SCD.

EPL29. Interest in genetic susceptibility testing for lung cancer: Seeking motivation to quit or an excuse to continue smoking?

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Genetic testing for susceptibility to common diseases is expected to become widely available in the near future, with the potential use to motivate individuals to adopt risk-reducing health behaviors (e.g., cigarette smoking). Present concerns are that high-risk results may diminish motivation either by promulgating fatalism about the benefits of risk reduction or lower risk results could unduly reassure individuals. Currently, there is little data to inform these discussions. We explore whether smokers' interest in a hypothetical genetic test for susceptibility to lung cancer is associated with their beliefs about the potential motivational effect of testing. Data are reported for 264 blood relatives, ages 18-55, of late-stage lung cancer patients recruited from three cancer centers. Relatives completed a telephone survey assessing a battery of psychosocial variables. The sample is white

(93%); half are female, half are the child of the cancer patient, 19% are college educated and 60% reported strong desire to quit smoking (7 on a 7-point scale). 61% of relatives indicated they would take the genetic susceptibility test. 65% reported an advantage of such testing would be that a high risk result could motivate them to quit smoking (PROMO), 21% reported a low risk result could motivate continued smoking (NEGMO), and 19% felt strongly that both could be advantages of testing. In multivariate analyses, perceived PROMO or NEGMO as an advantage of testing was significantly associated with greater interest in testing (Odds ratios, 5.1, 2.2, respectively). Implications for offering genetic susceptibility testing to motivate behavior change will be discussed.

EPL30. Prevalence of psychiatric disorder in pre-symptomatic HD gene carriers and non-carriers

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Psychiatric symptoms are a common feature of Huntington's disease (HD) and often precede the onset of motor and cognitive impairments. Examining the rate of psychiatric disturbances in pre-symptomatic individuals may help to determine whether such symptoms represent a prodromal manifestation of the disease, or whether they occur as a result of social and psychological adversity associated with a family history of HD. To date, the few prospective studies investigating psychiatric symptoms in pre-symptomatic at-risk individuals have only involved small samples of patients or have not used appropriate control groups.

The present study compared the prevalence of psychiatric symptoms in two groups of at-risk asymptomatic individuals (gene positive and gene negative) requesting predictive testing for HD between 1987 and 1999. Lifetime psychiatric histories of 204 at-risk individuals (89 gene carriers, 115 non-carriers) were obtained using the Composite International Diagnostic Interview (WHO, 1987). Lifetime and 12-month diagnoses were determined according to the DSM-III-R criteria of the American Psychiatric Association. Neither participants nor examiners were aware of gene status at the time of testing.

A preliminary analysis revealed no difference between gene-positive and gene-negative groups in the lifetime frequency of clinical psychiatric disorders. However, gene carriers did report a significantly higher 12-month prevalence of sub-clinical depressive symptoms. We will present the results of a further analysis currently in progress to investigate the relationship between psychiatric symptoms and proximity to onset of HD in pre-symptomatic individuals carrying the HD mutation. The findings have important implications for the underlying basis of psychiatric disturbances in HD.

EPL31. Premorbid aspects of personality as predictors of Huntington's Disease symptomatology

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Background: There is a considerable amount of phenotypic variation in Huntington's Disease patients. Little is known about the causes of this variation. In this study, we investigate associations between premorbid personality and clinical features in hospitalized HD-patients.

Method: We gathered information about premorbid personality, psychological and social characteristics of HD patients hospitalized in 5 specialized nursing homes in The Netherlands and Belgium. We interviewed contact persons who were close to them before they became symptomatic and we asked them to fill in questionnaires about the patient's premorbid personality (NEO-FFI), anxiety and depression (HADS), expectations about the future (BHS) and social support (SSQ). The patient's illness behaviour was assessed with the UHDRS and the BOSH*, additional information was extracted from the patient's medical record.

We performed a regression analysis to see if premorbid variables predict clinical features.

Results: Preliminary results indicate that patients who show more rigid and aggressive behaviour were premorbidly more anxious, more negative about the future and more introverted. Patients with more social-cognitive deterioration were premorbidly more anxious, more introverted, more open to experiences and had less instrumental support.

Discussion: In this group and based on retrospective data, there seems to be an association between aspects of premorbid personality and phenotypic variation in hospitalized Huntington's Disease patients. Within a life cycle approach our findings may be relevant for support of individuals and families.

*Timman et al, *Nature and development of Huntington disease in a nursing home population: The BOSH Rating Scale*, Cogn Behav Neurol. 2005 Dec;18(4):215-22.

EPL32. A qualitative study into the impact of Juvenile Huntington's Disease on the family.

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Objectives: To explore the issues faced by parent/guardian caregivers of young people with Juvenile Huntington's Disease (JHD)

Methods: Ten interviews were carried out with parent/guardian caregivers. Their experiences of caring for a child with JHD were explored, particularly focusing on their experiences of information, services and support they had received. The interviews were transcribed verbatim and analysed using the qualitative method Interpretative Phenomenological Analysis (IPA).

Results: One of the main themes emerging from the analysis of the interviews was the isolation that families felt, which appeared to be due to an engagement in social comparison with a number of different groups (e.g., adult-onset Huntington's Disease) and a sense of JHD as being different. This meant that the child with JHD and their parents were unable to normalise their experiences. This sense of isolation was exacerbated by a general lack of knowledge and understanding. Other themes describe how parents perceived the natural history of JHD. For example, parents found specific symptoms, such as challenging behaviour, particular difficult to cope with. Other themes presented included a definition of what families perceived to be helpful and unhelpful support.

Conclusions: A number of needs were highlighted by the families, which have implications for those providing support for families affected by JHD. There were also specific implications for the provision of support during the period before families receive a confirmed diagnosis, which families often highlighted as a particularly difficult time.

EPL33. Predictive genetic testing of minors on parents' persistent request; to whose advantage following an abnormal test result? A pilot study among six families

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Dutch clinical genetic centres generally follow the most undisputed criteria for testing minors such as: onset of the condition regularly occurs in childhood, (medical) interventions can be offered and / or the results will contribute to the counselling of family members. Reasons for not testing minors are based on psychosocial and ethical principles including insufficient emotional and cognitive development and / or young adolescents not yet being capable in moral reasoning to come to proper autonomous decisions.

Nevertheless, geneticists may choose to provide tests in children for non-medical reasons in specific cases, i.e. when severe anxiety in parents probably will harm the children. These parents are unable to cope with the uncertainty of not knowing and hope for normal test results for their minors.

We ask ourselves: how did families, counselled in our institute, cope with abnormal test results in their children if no expression of

the disorder is yet to be expected in childhood and no screening or preventive treatment is indicated?
Method: a follow-up of previous psychosocial counselling consisting of a semi-structured retrospective interview with parents one to seven years after the test result.
Preliminary conclusions and recommendations: It can be relevant for parents and their children to have time to cope with the diagnosis before the age of possible expression. Psycho-educational support according to protocol gives parents the opportunity to explore their anxiety, to reconsider timing of the test moment and to learn about their communication skills concerning heredity and the disorder.

EPL34. Living with Hereditary Cancer

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This qualitative study is a part of a larger study that aims to explore how individuals experience living with increased cancer risk, how this situation influence quality of life and to build knowledge about this patient groups in order to provide optimal care. Different high risk groups and patients suffering from cancer were included. An interview guide based upon Griffin focusing on quality of life, the emotional and practical implications of this condition, and participating in a clinical follow-up program was utilized. The first study describes how patients with MEN-1 experience their situation. The data were analyzed using Giorgi`s four step phenomenological approach. Four main categories and several subcategories were identified; The mixed feelings of being in a follow-up program, the effect of MEN1 upon daily activities, coming to terms with the condition, and uncertainty concerning the future. At the time of interview, none of these MEN-1 patients had received genetic counseling. Our findings indicate that a majority of patients have adjusted to their situation, describing themselves as being healthy despite physical and psychological symptoms and treatment. They report a shift in priorities after developing MEN1 or learning about their personal risk. The participants received decent care in the clinical follow-up program, - however, greater effort should be put into patient information.
These patients might benefit from genetic counseling that aims to achieve empowerment by mobilizing and strengthening the patients' resources. Health professionals involved should recognize their potential impact and influence on patient's ability to adjust to these circumstances.

EPL35. Long-term Follow up of lifestyle changes in persons attending genetic counselling for breast, ovarian or colorectal cancer

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Purpose: The aim of this study was to explore long-term changes in lifestyles and relationships after receiving oncogenetic counselling. Another investigated aspect was how people felt about the genetic information.
Patient and methods: A total of 307 persons who attended genetic counselling at the onco-genetic clinic of University Hospital in Uppsala, Sweden (1998-2002) were asked to participate in a long-term follow up, 3-7 years later. There were 197 female and 18 male (215 in all) who answered the questionnaire (including self-reports of life-style changes) and 158 who estimated their perceived risk.
Results: The majority was satisfied with the information and felt that the estimation of their risk was not surprising and that it was better to be informed of the risks. The information had changed the majority's view of the future and they felt that now they appreciate the every day life more. Approximately half of the participants reported that the information had a strong effect on their lives and on their relations with their family. A few reported changes in priorities with regard to work and education after getting the information.
Persons with high-risk estimation reported stronger impact on their lives and view of the future, as compared to those with perceived low risk.
Conclusion: Most participants were positive to receiving information about their genetic disposition. However, a minority experienced negative changes in their lives. Changes in family relations were relatively common. Assessed variables such as life-style and health behaviour were seldom related to perceived levels of risk.

EPL36. The course of distress in women at increased risk of hereditary breast and ovarian cancer who opt for prophylactic surgery

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Introduction: Prophylactic mastectomy (PM) and prophylactic salpingo-oophorectomy (PSO) are the most effective options to obviate the increased risk for breast and ovarian cancer, respectively, in case of a hereditary predisposition.
Objectives: The present study investigated the levels and the course of psychological distress before and after PM and/or PSO in 78 women with an increased risk for breast and/or ovarian cancer due to a BRCA1/2 mutation or a hereditary predisposition.
Methods: The Hospital Anxiety and Depression Scale (HADS) measured general distress and the Impact of Events Scale (IES) assessed cancer-related distress at baseline (2 to 4 weeks before prophylactic surgery), and 6 and 12 months post-surgery.
Results: Women who opted for prophylactic surgery had higher distress levels prior to surgery as compared to the distress levels in a reference group of women who opted for regular surveillance. The levels of distress in women who opted for PM did not significantly differ from the distress levels in women who opted for PSO, at both baseline and follow-up. However, women who opted for PM showed a significant decrease in anxiety and cancer-related distress after surgery, whereas the course of distress in women who opted for PSO showed no changes. Finally, a substantial amount of women experienced clinically high levels of cancer-related distress and anxiety one year after prophylactic surgery.

EPL37. Creating the link. Patient stories promoting engagement in genetics: a web-based resource.

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Stories provide common ground on which patients and professionals can engage with real-life problems. 'Telling stories, understanding real-life genetics' is a multi-media web-based resource for healthcare professionals which will encourage the understanding of genetics, its impact on peoples' lives, and relevance within non-genetic specialties. Hosted by the NHS National Genetics Education and Development Centre, the site will be launched during 2006 with free access.
Following ethics approval, recruitment to this educational project has been via support groups, community organisations and conferences. More than 60 stories have been collected in written, audio and video formats from patients, carers and practitioners. Minority groups and people under 30 years are currently under-represented. Story content has been mapped to the genetics competency framework (Kirk et al. 2003), and this approach validates the framework's development, highlighting areas requiring improved education and training.
These stories will be used to enhance a genetics educational 'tool-box' based on the competency framework. Designed to be used by both educators and learners, the resource will be searchable and will provide structured teaching and learning objectives. Each story will contain links to additional information. This could include notes for further explanation; points for reflection and discussion; implications for

professional practice; suggested activities to help the reader develop competence, and contact details of relevant support groups.
Initially developed for nurses, midwives and health visitors, it is anticipated that this resource will be of value across the healthcare professions, and use by other groups will be encouraged.

EMPAG Workshops

EW1. Narrative workshop: an approach to explore meaning in the context of genetic counselling

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Most definitions of genetic counselling include the aim of helping counselees adjust to their genetic situation. There is some research evidence to suggest that it is the personal meaning constructed by patients that is important in understanding adaptation.
This workshop will draw on the cognitive-narrative model as a way of exploring how patient's stories can be utilised more fully in the context of genetic counselling. The theoretical assumptions underpinning the model will be outlined. We will consider what can be learnt from the way patients construct and give meaning to their episodes and how patients can be guided to explore and create new meanings for genetic information.
Case examples will include narrative techniques used by one of the authors (JR) to support women in their adjustment process following termination of pregnancy. There will be the opportunity for participants to try some of the techniques that may be particularly useful in genetic counselling practice including metaphor generation; a creative way of providing awareness of meaning content and alternative versions of a particular event or episode. This exercise is guided through a structured manual and the authors will address any specific difficulty raised from ongoing exercise.
The relevance and applicability of this framework is discussed in what concerns limitations and adequacy.

EW2. Using meta-ethnography to synthesise qualitative research

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Qualitative methods are increasingly used alongside quantitative methods in primary research on the grounds that the two sets of data can be complementary, but methods for incorporating qualitative research in systematic reviews are relatively under-developed and under-evaluated. They present a major methodological and practical developmental challenge.
Objective: This workshop will introduce participants to the principles and practice of qualitative systematic reviewing and synthesis using the approach of meta-ethnography.
Content: A short, didactic presentation and handout will provide an overview of qualitative research synthesis in general, and meta-ethnography in particular. The presenter's recent systematic review and synthesis of lay understanding of familial risk of common chronic diseases will be used to illustrate the theoretical framework of meta-ethnography. Extracts from qualitative papers will be provided for participants to practice appraisal and synthesising skills in small group activities. The small groups will share the results of their work with the larger group, and open discussion will be encouraged to elicit specific problems and challenges.
Prerequisite Knowledge: An interest in qualitative research methods.

EMPAG Posters

EP01. The dilemmas of the high-risk pregnant women - assisted by a public genetic medical care in Rio de Janeiro/Brazil

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The study aimed to investigate the interactions and attitudes of the high genetic risk pregnant women facing the prenatal diagnosis in a public clinical care in Rio de Janeiro, Brazil. Methodology: To collect data was used the ethnographic perspective and Life History intervew: There were total 1200 hours of observations in the genetic medical assistence and 14 women were intervewed. the analisis of the material (pregnant women dopoiments) was made according to the psychoanalitic and social-cultural approaches. The pregnant women was sent to the prenatal diagnosis service according the following criteria: advanced maternal age; family history of genetic disease and complications in the current pregnancies that sugested a genetic cause. Conclusions: To most women investigated, the prenatal diagnosis is an experience marked the by afective, legal and familiar helplessness and could be seen as an anxiety *panoptic*. Therefore, the effectiveness of the medical procedure addopted in the prenatal diagnosis does not lie in the defective gene cure, but in the possibility of the patient to deal with the genetic risk idea and produce a new meanning about the risk pregnancy and about the child to be born.

EP02. Long-term psychosocial impact of familial adenomatous polyposis (FAP)

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Background: Familial adenomatous polyposis (FAP) is a hereditary condition characterized by the development of a large number of polyps in the colon. Without surgery the large majority of individuals will develop colorectal cancer by the age of 45 years. Individuals from high-risk FAP families are offered genetic testing from the age of 10 years onward.
Purpose: To investigate the long-term psychosocial impact of FAP on individuals from high-risk families and on the family.
Patients and methods: This nationwide cross-sectional study will invite all individuals from FAP families to complete a self-report questionnaire (estimated n=750), followed by a semi-structured interview with a subsample of respondents (n=60). The questionnaire assesses surveillance behavior, experiences with genetic testing and preventive surgery, physical symptoms, a range of psychosocial measures and the perceived need for professional psychosocial support. Additional questions assess attitudes toward prenatal diagnosis and pre-implantation techniques. Partners of individuals with a clinical or genetic diagnosis will complete a subset of these questions. Data from a pilot study highlighted problems with relationships, sexuality, fatigue, feelings of guilt and psychical complications and long-term effects of surgery affecting daily life.
Relevance: This study will contribute to our understanding of the long-term psychosocial impact of being a member of a family at high-risk for FAP, and will provide insight into (not) undergoing genetic testing, surveillance, and risk reducing surgery among FAP family members. This information can be used by health care providers for counseling and psycho-education programs for individuals at high risk of developing FAP.

EP03. Prenatal testing - Can we predict or influence on women's choices?

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Introduction: Utilization rates of prenatal tests vary with the type of test. Compared to ultrasound scans and maternal serum screening, uptake rates of amniocentesis and carrier screening for genetic diseases are generally lower.
Objectives: To identify factors associated with utilization of amniocentesis and carrier screening.
Methods: The study was conducted in southern Israel in which 596 Jewish women, who had given birth at Soroka University Medical Center, were interviewed by phone using a structured questionnaire, at 5-8 weeks postpartum. Of them, 464 (77.8%) agreed to answer a knowledge questionnaire.
Results: Rates of test uptake were 22.2% and 20.8% for amniocentesis and genetic tests, respectively. In a multivariate logistic regression

performed on the 464 women who answered the knowledge questionnaire, factors associated with carrier screening were secularism (OR = 3.032), high levels of knowledge regarding prenatal tests (OR = 3.917) and satisfaction with pretest counseling (OR = 7.177).
The same factors were associated with uptake of amniocentesis (ORs = 3.834, 2.301, 6.484, respectively), with the addition of age ≥35 years (OR = 2.674), medical recommendation for the test (OR = 2.822), and previous termination of pregnancy (OR = 6.087).
Conclusions: Accepting a prenatal test is associated with religious and cultural beliefs, but also with a more informed decision than the declining of one. While religious and cultural aspects of prenatal decisions should be respected by health providers, strengthening lay knowledge regarding prenatal tests and improving professional counseling will allow more informed choices, which might result in higher uptake of prenatal testing.

EP04. An evaluation of a shared experience group for women following prenatal diagnosis and termination for a fetal abnormality.

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Support After Fetal Diagnosis of Abnormality (SAFDA), is a facilitated shared experience group for women in Victoria, Australia who have had a termination for a fetal abnormality. SAFDA is held at a tertiary maternity hospital, and five, two-hour groups are held each year with approximately 4-5 persons attending. Partners, other family and support people are welcomed. The facilitators are a social worker and a genetic counsellor.
A questionnaire-based study was undertaken between 2001 and 2005 to evaluate SAFDA. The de-identified questionnaire included questions relating to the referring professional, participants', prior expectations of the group, preferred group format, and rating of helpfulness of participation, length and venue. There was also opportunity for participants to comment generally on their experience in the group.
A total of 85 participants (100% response) completed the questionnaire. The main referring health professional was a genetic counsellor (64%). Seventy-one participants (84%) considered it 'very helpful' to participate in the group. Seventy-eight participants (92%) considered that a shared-experience group was the most beneficial format. Seventy-eight participants (92%) considered that the size of the group was 'just right'. Comments written by participants affirmed that the present format of SAFDA was a highly valued opportunity to listen to and share experiences in a confidential small group.
This study highlights the importance of providing women who receive a diagnosis of fetal abnormality during their pregnancy with an appropriate forum to share their experiences, and provides useful information and insights for health professionals who are considering how best to support the women.

EP05. Attitudes of men with Becker Muscular Dystrophy towards genetic testing

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There is a considerable body of research into women's attitudes towards and experiences of genetic testing. A limited number of studies have focused on men, and few have involved men affected by an inherited condition. Semi-structured interviews with ten men affected by Becker Muscular Dystrophy (BMD) were undertaken to gain an insight into their views about women undergoing carrier testing and prenatal diagnosis for the condition, and on pre-symptomatic testing of at-risk boys. They were also asked about their thoughts about having children themselves.
The key themes that emerged with regard to their attitudes to carrier testing and prenatal diagnosis were that they identified the importance of women being able to make informed reproductive decisions, and an understanding that women might feel guilt about passing on the condition. Perceived severity of BMD, capacity to cope and individual choice were seen as important factors in reproductive decision-making. Their opinions about the appropriateness of prenatal diagnosis and termination for BMD varied, and were influenced by beliefs about the value of life, attitudes to disability, and reflecting on their personal experiences; whatever their beliefs and experiences, all the men valued their own lives. Issues of concern around pre-symptomatic testing included timing, benefits and disadvantages of testing and potential differences between the parents' and child's outlook. Factors that influenced their own reproductive decisions included their daughters having to make difficult reproductive choices, the chance of affected boys in future generations, the physical limitations of the condition, and the possibility of advancement in medical research.

EP06. Making decisions about amniocentesis

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Many pregnant women in Victoria, Australia have a Maternal Serum Screening (MSS) test either in the first or second trimester of pregnancy. These screening tests can indicate an individual's risk of having a baby with a chromosomal anomaly or a neural tube defect.
Women who receive an 'increased risk' result are generally offered genetic counselling in order to provide information about their test result and the options available for diagnostic testing such as amniocentesis or CVS. In these circumstances genetic counselling aims to facilitate informed and autonomous decision-making (Hodgson & Spriggs 2005) but due to a lack of process studies there is currently little known about how women experience this phenomena or indeed how genetic counselling might achieve this aim.
As part of a PhD project entitled "Women's experiences of prenatal genetic counselling" 21 'increased risk' genetic counselling sessions and 15 follow up interviews were audio-taped and transcribed. Thematic and content analysis provided:-
a)
an insight into what actually happens in prenatal genetic counselling
b)
rich descriptions of how pregnant women in this cohort experience being at increased risk for a chromosome anomaly.
Using contrasting individual accounts this presentation explores selected women's experiences of prenatal genetic counselling. These accounts illustrate differences in the methods and outcomes of decision-making processes and highlight the utility of genetic counselling as a means to facilitate informed decision-making in the prenatal setting.
Hodgson J & Spriggs M. (2005) Journal of Genetic Counselling. Vol 14, No 2 pp. 89-97

EP07. Genetic prenatal diagnosis - Psychological aspects and reproductive behavior of families

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AIM: study of the influence of the genefic prenatal diagnosis on the emotional state of the patients; significance of medical and informational factors on the reproductive decision of the family.
METHODS: information interview; anxiety questionnaire; grief scale.
MATERIAL: 120 pregnant women: 30 after US screening; 30 because of positive family history, 30 - increased maternal age and 30 healthy controls.
RESULTS: Women with previous reproductive failure and those who had higher educational level proved to be well informed. Most of the women have been information by a gynaecologist or clinical geneticist. High proportion of tested women consider reliable the diagnostic and prognostic potential of the prenatal genetic diagnosis. The type of procedure influences insignificantly the reproductive decision.
CONCLUSIONS:
1.The reproductive decision depends on the quality of genetic counseling.
2. The level of anxiety is determined by the kind of procedure and reproductive history.
3. Age, previous loss, the type of the loss are decisive factors for coping with the loss.

EP08. Risk perception of pregnant women being offered prenatal screening.

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Ideally, the decision for or against prenatal genetic screening should be based on the active decision-making of pregnant women who should be fully informed in particular about the risks involved. In a large randomised controlled trial (N=2785) with two groups being offered prenatal screening and a control group, we measured pregnant women's perceived risk of giving birth to a child with Down's syndrome. This was measured with a numeric risk scale and recalculated into three categories: (1) a risk between 1 out of 2 and 1 out of 200; (2) a risk between 1 out of 200 and 1 out of 1000; (3) and a risk smaller than 1 out of 1000. Participants generally perceived their risk as rather low: about 77% of the women thought they had a risk below 1 out of 1000 before information was offered while this was about 43% after test information was given as compared to 72% of women in the control group (a significant difference χ^2 (4) = 162.8, p <.001). Before the test offer, about 50% of women correctly classified their risk. After the offer but before the test was done, significantly more women classified their risk correctly compared with the control group (62.1% versus 54.5%; $\chi^2(2)$= 9.90, p < .01). Although there is an increase in accurate risk perception, it is worrisome that still a large number of women had an inadequate perception of their risk of giving birth to a child with Down syndrome.

EP09. Risk unawareness and psychological consequences of prenatal diagnosis in a case of familial Angelman Syndrome

E. Razzaboni[1], *D. Turchetti*[1], *H. Zomer*[1], *S. Ferrari*[1], *D. Greco*[2], *C. Graziano*[1], *G. Romeo*[1], *M. Seri*[1];
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Angelman Syndrome, a disorder characterized by mental retardation, absence of speech, seizures and motor dysfunction, caused by loss of expression of the maternal copy of the UBE3A gene, is generally sporadic. However, familial cases may occur as the result of mutations in the UBE3A gene or of the Imprinting Center. We describe the case of a pregnant woman having two nephews with Angelman syndrome caused by a UBE3A mutation; due to poor communication within the family, she was completely unaware of this diagnosis and of the subsequent risk of recurrence until 15 weeks of gestation. Mutation analysis in the woman revealed she carried the familial mutation. Amniocentesis and prenatal genetic test were then performed, demonstrating that the fetus had inherited the disease. The pregnancy was terminated at week 21. The unexpected diagnosis and the subsequent pregnancy termination caused the woman a severe psychological distress showing relevant psychopatological symptoms and psychological support was immediately offered. At two-year follow-up she showed a better adaptation to grief. The unawareness of the Angelman Syndrome risk appeared to have significantly influenced her adverse psychological reaction. Considering this factor, it could be useful prompting future researches toward a deep comprehension of the relation between risk awareness and psychological impact of fetal anomalies communication. Furthermore, medical and psychosocial support received from professional caregivers were of great value for the woman and helpful in her grieving process according to her reports. This led us considerate that a multidisciplinary professional support should be always given in cases like that.

EP10. Difficult choices. Patient experiences with genetic counselling before prenatal diagnostic testing.

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The purpose of the present study was to put forth genetic counselling experiences for pregnant women at risks before prenatal diagnostic testing. The aim has ben to get patients to describe their experiences and if this experience has influenced them in the present situation.
The empirical basis is built on interviews of nine woman, 38 years or more by expected time of birth. All were recruited from an institution approved to give genetic counselling. The interviews were carried out after the woman had been to genetic counselling but before a prospective amniocentesis. Some had been to ultrasound in first trimester where parameters for chromosomal deviations were assessed.
A phenomenological-hermeneutical approach has been used where genetic counselling experience and possible consequences have been focused. Genetic counselling and care have been used as theoretical perspective.
The woman experienced genetic counselling as positive, relieved and reduced anxiety. At the same time all patients except one did put forward that the offer, genetic counselling and prenatal diagnosis testing had an impact on them, increasing ambivalence, dilemmas and difficult decisions of existential nature.
The studiy results form the basis of underlining the importance that counselling has to be imbued with the principles of genetic counselling and the concept of care has to be an important part of this.Evaluation and reflection on the content and quality of the counselling is important and necessary.
Findings imply the necessity for futher studies.

EP11. Addressing the Psychosocial Aspects of Research on Lethal Birth Defects: A Call for Research

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Despite recent advances in genetic technology, little is known about the etiology of lethal birth defects like anencephaly. The study of these birth defects is particularly difficult given that many pregnancies are terminated shortly after diagnosis. As such, one proposed method involves utilizing genetic counseling resources to enroll patients into a study within days after diagnosis, which is critical to minimize recall bias of exposures and to obtain tissue samples for genetic analysis. One ethical concern of using this method is that participation may pose a significant emotional burden for the parents. Potential benefit to parent participation might include an opportunity to discuss concerns in a supportive environment, emotional support and a decreased sense of isolation, and a feeling of altruism in contributing to scientific research. Although it is possible to extrapolate from studies addressing the ethical and psychological impact of research participation within bereavement and trauma-based settings, there is no literature on the emotional repercussions of parental participation in studies focusing on lethal birth defects. There is also a paucity of literature that examines the psychosocial implications of surveying an individual directly after a sensitive event has occurred. This paper will provide a detailed account of the existing literature and make suggestions for areas where future empiric research would be beneficial.

EP12. Prenatal screening for Down syndrome: Are women getting what they want?

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Ideally, a woman should have educational support, time and opportunity to consider her screening options, discuss ethical issues with her partner and others and feel confident in making an informed choice that is relevant to her own personal / family beliefs and current situation. How often does this happen in reality?
The relatively rapid evolution of technology in recent years has led to an array of prenatal screening tests being offered in clinical practice, and women are sometimes expected to make a decision in a brief timeframe. Both women and their healthcare providers are often left confused by the complexity of new options. Questions are often raised by the media and consumers, as to the apparent 'routine' nature of these tests, the way the options are presented by healthcare professionals and what women really want.
A two-part pilot study was undertaken at the Mater Mothers Hospital, Brisbane. Around 300 women (public and private patients) were surveyed to examine the reasons why pregnant women either have or do not have a prenatal screening test for Down syndrome, and to compare their demographic, obstetric and information-seeking characteristics. The women's knowledge of prenatal genetic counselling services was also examined. In the same time period, around 150-200 midwives, obstetricians, registrars and family physicians were surveyed to examine the healthcare professionals' current knowledge, attitude and practice with respect to prenatal screening for Down syndrome.

EP13. Communicating genetic information to family members- what are the guidelines telling us?

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International guidelines recommend that people who undertake genetic tests should inform their relatives if the results have implications for them. Experience indicates however that many people do not pass on that information to their relatives.

The authors are investigating families' experiences of communicating genetic information and whether there is a role for genetic health professionals within this experience. Three stages of the project are planned including interviews with probands and their family members and a survey of health professionals. Some qualitative preliminary data has been collected, involving individuals with a genetic condition.

This paper describes results to date of this research and considers the obligations of genetic health professionals who become aware that family members have not been informed about their potential risks. An analysis of the guidelines has been undertaken demonstrating the differences that exist and the variations in approaches taken between national, regional and international settings. The data illustrates the gaps in evidence about how health professionals interpret the guidelines for problems that arise in their practice involving family communication.

EP14. Talking about colorectal cancer risk - family influences and dynamics

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Genetic counselling for colorectal cancer risk is offered to unaffected men and women at increased risk to warrant screening. However their family history may not fulfil high risk criteria. This study looked at the experiences of individuals with no genetic 'label' to explain the family history. In depth interviews were conducted with those previously seen for genetic counselling. Interviews were transcribed and thematic analysis highlighted areas for consideration, in particular lines of communication within the family and responsibility for relatives. Family communication is not straightforward, generational and sibling differences combined with responsibility and the need to protect relatives was complicated by some having had cancer. The results demonstrate the strong desire within these families to avoid upsetting relatives whilst encouraging them to be proactive about preventative strategies. Complex relationships within families were evident showing the importance of understanding the family structure. Some participants shared their feelings with the family, for others disseminating information or just talking about the risk was difficult. Health beliefs had a strong influence in relation to perception of screening and the importance of diet and a healthy lifestyle combined with social taboos relating to bowel disease. None of the participants had confirmation that the family history of cancer was due to a genetic predisposition, however responsibility for close relatives was still very important to them. In order to conduct counselling that maximises psychological adjustment of family members, consideration of family dynamics is essential.

EP15. The Importance of Communication and Experiential Knowledge in Hereditary Nonpolyposis Colorectal Cancer

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Hereditary Nonpolyposis Colorectal Cancer (HNPCC) is characterized by increased risks for several tumor types with the highest risks for colorectal cancer (80-90% life-time risk) and endometrial cancer (40-60% risk). Predictive genetic diagnosis for cancer has evolved during the last decade and is enforced since control programs for at-risk individuals detect tumors earlier and thus reduce mortality, but knowledge about how this new information affects the life situation is limited.

We performed oral, tape-recorded interviews with 10 Swedish carriers of high-risk alleles for HNPCC. The interviews were analysed through inductive content analysis and the results demonstrate the impact of the communication processes and the importance of the individual's previous experiences from cancer. Individuals in HNPCC families have often experienced early onset cancer in close relatives. The responsibility for informing family members about the hereditary cancer within the family is perceived as a burden, but is facilitated by an open communication and good contact within the family. Experiential knowledge influences coping possibilities and may strengthen as well as weaken these; most individuals who have previously reflected about the possibility of hereditary cancer perceive the information about a genetic change as a confirmation of their suspicion, whereas those for whom the knowledge is unexpected seem to need more support. In order to reduce stress and negative reactions in the latter cohort, we suggest that future studies should aim at identifying those individuals in a family who need extended support.

EP16. 'Oh this is to do with me': Communicating with children about newborn screening results.

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Background: Newborn screening for cystic fibrosis in the UK raises questions regarding how and when people identified as carriers should be informed. A gradual communication process beginning in childhood has been suggested as optimal, although little research has addressed how children understand such information.

Objective: To examine genetic counsellors' experiences of counselling children in order to assess how best to communicate with children about genetics.

Methods: Study packs were distributed to all 21 UK Clinical Genetics Centres inviting Association of Genetic Nurses and Counsellors members to participate in a semi-structured telephone interview.

Results: From the 28 interviews, themes central to communicating with children about genetics were identified. Facilitating autonomy was viewed as fundamental in counselling children. Additionally, personalisation of information and turning abstract concepts into concrete knowledge were important strategies to enhance comprehension.

Counsellors spontaneously identified barriers to communication, of which the taciturn child was the most common. Poor family communication and lack of materials for children were also named as barriers. Maturity, illness experience, and good family communication were perceived as facilitating counselling. Likewise, school education and the presence of supportive parents were cited as having positive effects.

Conclusions: The results illustrate that although genetic counselling of children is possible, a number of factors affect its success. Most are amenable to intervention, although their relationship to comprehension may be complex. Further research on communicating with children about genetics and the development of age-appropriate educational resources is vital to enable children to utilise the information screening programmes provide.

EP17. Balancing interests in the disclosure of genetic test results to relatives: An empirically based analysis

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Genetic test results can have important implications for relatives of the tested patient. Consequently, when the patient does not wish to have the test results disclosed, there is a significant ethical tension between the interests, rights and obligations of the patient and the interests, rights and obligations of the relatives. To our knowledge, policy analyses of this issue have not incorporated empirical studies of attitudes of the general population.

This presentation analyzes the policy implications of a recent survey conducted in Norway. A random sample of subjects (N=1077) were asked to indicate their perceptions and attitudes regarding disclosure of test results to relatives, with and without patient consent. These attitudes were correlated with attitudes and perceptions about other aspects of genetic testing.

Preliminary results show a tension within individuals regarding disclosure. There was a tendency to want to know the results of others, while not wishing to disclose their own. Further, there are indications of a possible link between wanting to know the results against the patient's wishes and issues of access. The author analyzes the empirical results within the context of interests, obligations, and rights to inform a more nuanced understanding of the issue, and to identify possible links to other aspects of the genetic testing experience. Taken together, examination of these links may indicate possible policy approaches to relieving some of the tension associated with the disclosure decision.

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EP18. Increased risk of breast cancer: do women really want to know?

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Background: The Netherlands Cancer Institute began its Family Cancer Clinic in 1995 to inform people about their possible increased familial and/or genetic risk of cancer. In the ensuing 10 years, we have observed that approximately 35 % of the clinic attendees prematurely discontinue the counseling process. The aim of the present study was to investigate the problems that attendees experience with starting and/or continuing genetic counseling.

Methods: Self-report questionnaires were sent to women who, during a period of 15 months, applied for genetic counseling for the familial occurrence of breast cancer but discontinued the counseling after their first contact. The questionnaire was sent, on average, 18 months after their first contact with the clinic.

Results: Of the 73 eligible women, 48 (66%) returned a completed questionnaire. The main self-reported reasons for discontinuing the counseling were: difficulties in anticipating the consequences of genetic counseling (28%), worries about not being able to cope with an unfavorable test results (20%), not having sufficient information about the occurrence of cancer in the family (20%), and wanting to postpone the genetic counseling until a later date. None of the respondents indicated negative initial experiences with the family cancer clinic as a reason for discontinuing the counseling. .**Conclusion:** During the initial contact with a family cancer clinic, sufficient attention should be paid to inquiring about clients' worries and concerns, and to informing them about ways that are available to cope effectively with test results, both positive and negative.

EP19. Genetic counseling during pregnancy: causes and consequences

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Reproductive genetic counselling preferably takes place prior to conception. In the Netherlands, however, an estimated 15 % of the people attend a department of clinical genetics for the first time while already pregnant. If we want to stimulate preconception genetic counselling for familial conditions, it is important to gain insight in reasons for the timing of referral. The main aim of our study, therefore, was to explore factors that may affect this timing. A second aim was to address some consequences of the timing of referral for the quality of the initial clinical genetic consultation.

Pregnant (n=100) and non-pregnant (n=100) women who visited our department of clinical genetics completed a questionnaire before and after the initial consultation. The counsellors completed a post-visit questionnaire. The consultations were tape recorded and analysed. In addition, a questionnaire was sent to the general practitioners of the pregnant women.

We found pregnant women to be less inclined to initiate genetic counselling themselves, probably because they estimated their genetic risk as lower and worried less. Most general practitioners were unaware of a genetic risk factor in their patient before the pregnancy. Finally, we found no important adverse effects of the timing of genetic counselling on the content and affective tone of the counselee-counsellor interaction during the initial consultation, nor on women's satisfaction.

Based on our findings, an active role of general practitioners and the implementation of a routine preconception consultation for every woman with reproductive plans are advocated.

EP20. Internet paternity testing in Italy: is it a good practice?

***P. Tasinato**, L. Caenazzo, P. Benciolini, D. Rodriguez;*

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Paternity analyses carried out by laboratories via Internet involve sending the requesting parties a kit for collecting samples followed by sending back the samples to the laboratory which will conduct the analyses.

Information are given to the subjects via the laboratory's web site or by the mailing of written notification.

Not complicated analyses are involved. However, the repercussions of the result have a great emotional impact that could, when unexpected, cause turmoil among the people involved, the greatest repercussions will inevitably be felt by the children.

Recently easy-to-use kits have also been introduced in Italy.

The Italian Privacy's Garante is assessing the ethical and legal implications but regulations are not yet in place.

In this work, we want to consider some legal and ethical issues linked to the method of investigation.

We will analyze problems relating to information, consent and certifying the origin of samples by way of outlining the relevant Italian deontological laws and codes, and the Oviedo Convention's information. We believe that adequate information on this subject cannot be produced via the Internet and, consequently, any such consent is not valid. Finally, we will analyze issues regarding the competence of the personnel who carry out analysis and the reliability of the laboratories involved.In our opinion, the complexity of the situations and expectations linked to paternity investigations require a special sensitivity in dealing with each case, each with its own specific legal and ethical-deontological issues, while taking into account the emotional stability of the subjects involved

EP21. Discovering misattribuitted paternity in genetic conseling: ethical and pratical issues

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Nowadays, the frequency of children identified as being biologically fathered by someone other than the man who believes he is the father is such that, in the field of genetic counseling, is likely to encounter cases of false paternity.

In the field of counselling, information is dealt with which is not requested by patients nor expected by them. This information, which seems to regard only the "father" of the family, actually has repercussions for the entire family. By now, it has been accepted that genetic information is by its nature both individual and familial.

When dealing with false paternity, as revealed through genetic counseling, what should the geneticist's course of conduct be? In accordance with the ethical principles and directives contained in the Oviedo Convention, must he/she reveal information in keeping with his/her duty to inform patients about the state of their health? Otherwise, must he/she refuse to supply such information in light of the negative repercussions and difficulties in managing them within the limited scope of genetic counseling? Should the principle be valued that a person has the right to such knowledge or should family unity and the serenity of its internal relationships be safeguarded instead? If a geneticist were allowed to make his/her own decisions in individual cases, which criteria should guide him/her? Does the danger of geneticists' paternalism exist?

This work offers a reflection on behavior in cases of disclosure of misattributed paternity, as based on ethical principles also according to the Oviedo Convention.

EP22. An exploration of genetic professionals' perceptions of non-directiveness in genetic counselling consultations

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As yet there still seems to be a lack of agreement, amongst those involved in clinical genetics, regarding what actually constitutes non-directiveness. This study aims to explore the perceptions and understanding of genetic professionals relating to non-directiveness and its continued appropriateness in genetic counselling. Loosely structured focus groups with eight clinical geneticists and genetic counsellors studied their recognition and understanding of non-directiveness, as well as how appropriate they believe non-directiveness is, and how non-directiveness influences their practice. Analysis of transcribed focus groups revealed that participants had the following understanding of non-directiveness: Not influencing clients and letting them make their own decisions by; presenting all aspects of a scenario, not giving an opinion, not being overtly influenced by ones own values, internally monitoring choice of words and non-verbal signals and responding to clients' cues on a case-by-case basis. These results may have implications for any future attempts to provide an operational definition of non-directiveness that is collectively agreed upon by genetics professionals.

EP23. Concepts of Genetic Counselling

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Many studies show that after a positive diagnosis of Down's syndrome, 70 to 100% of couples choose selective abortion of the affected fetus. For example, among the first 7,000 women receiving prenatal diagnosis under a public health programme in New York City, 97% aborted for Down's syndrome.

When deciding to have the procedure, parents-to-be use several sources of information: the referring physician or genetic counselor, written and electronic media, and other people who had the procedure. The same sources of information are present when the parents have had the procedure and are expecting and receiving results of amniocentesis. The news that the fetus is affected with a disorder has even more profound consequences as the couple grapples with the issue of whether or not to continue the pregnancy.

In this paper, we intend to address the issue of genetic counseling after a positive diagnosis of Down syndrome from two aspects. The first aspect is narrow counseling, which includes information about physical and psychological characteristics the child is expected to have, as a person affected with Down's syndrome. The second aspect is wider counseling, which, along with the medical information, includes experience of parents already raising children with Down's syndrome. To reach a fully informed choice on a matter important as this, these two approaches should be equally considered.

EP24. Psychosocial barriers to uptake of clinical services for hypertrophic cardiomyopathy; implications for service design

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The provision of services for families with hypertrophic cardiomyopathy (HCM) has been evolving with the advances in detection, management and genetic analysis of this condition. The advent of genetic analysis, in particular, brings with it the potential to clarify risk status for first degree relatives and to offer a health economic advantage. However, uptake of clinical screening with or without genetic testing is not one hundred percent amongst at-risk family members. Psychosocial influences such as chronic anxiety, positive or negative family role models, and readiness to confront risk status may extend this process over months or years. We present three cases illustrating how psychosocial issues prolonged uptake of clinical services and required flexibility on the part of clinic providers. These issues are more the exception than the rule and are more likely to occur in families who have suffered bereavement due to HCM. Our experience supports the presence of a healthcare provider designated to handle and keep track of psychosocial issues arising in families with HCM, minimising the chance that individuals requiring more time are lost to follow-up. We describe the Oxford Genetics Knowledge Park model of service involving a genetic counsellor which has been implemented over three years. Features of this service include particular attention to counselling pre-clinical assessment, and strategies to help families with cascade information and screening. Case studies of psychosocial barriers to uptake of clinical services for HCM are important to inform the development and assessment of services for families with inherited cardiomyopathies.

EP25. A comparison of different care pathways in two British Genetics Centres

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Patient satisfaction when attending as a result of a referral for Marfan syndrome was examined in two British Genetics centres with different models of care. Clinic 1 offered a one stop Marfan clinic. Clinic 2 offered a traditional genetics appointment with subsequent referrals as required.

The literature indicated that the multidisciplinary clinic may be beneficial for patients and health care professionals in terms of efficiency of service and the pooling of resources. Little data exists on patient satisfaction in different models of care.

Patient satisfaction was measured using an audit tool for genetics service (Skirton et al, 2005). 25 patients responded to the postal questionnaire, giving a response rate of 49%. There was a statistically significant correlation between overall satisfaction and the outcome measure, greater peace of mind (Pearson's correlation coefficient 0.007). Patients in Clinic 1 ranked outcome measures related to a diagnosis being made or refuted higher than Clinic 2 patients. Clinic 2 patients ranked outcome measures related to understanding the condition higher than Clinic 1 patients.

It may be suggested that patients in Clinic 1 achieve 'greater peace of mind' because more of them are told in one clinic appointment whether they have Marfan syndrome or not.

It is important to consider that making a diagnosis is one aspect of a genetics referral. The experience of patients given the diagnosis of Marfan syndrome in the one stop clinic requires further investigation.

EP26. Ideal genetic counselling in international guidelines

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Background: In order to recognise the societal implications that are produced within counselling in the context of genetic testing, there is a need to study the ideals that are directed towards it. Guidelines for counselling can be considered to reflect these ideals.

Objective: To review how genetic counselling is defined in international guidelines and to examine what kinds of expectations and ideals are concentrated on it, after which the ideology behind the ideal counselling can be investigated.

Methods: Guidelines for genetic counselling that have been produced by international and European political bodies, regional professional organisations, ethical boards and patient associations were collected and classified using the computer-aided analysis programme QSRNudist. The classifications were analysed with discourse analysis that focuses on the uniform mechanisms of meanings. The objective was to find the collectively accepted conceptions of genetic counselling.

Results: In the guidelines, counselling is seen as a unique process of providing very special information. The speciality of genetic information is a core issue, which creates the basis of ideal counselling. The information is expected to be too complex for ordinary people to understand without the help of specially trained professionals and particular methods. The ideal counselling concentrates on individual choices, whereas family is seen as a problem that endangers the values of individuality and confidentiality. In the constructions of ideal counselling a well-trained professional counsels people on their individual situations, telling all the facts honestly, but empowering their own evaluations and ethics.

EP27. Application of a specific instrument to evaluate the level of anxiety (SAT) in 16 families with a diagnosis of triple X in their daughter

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Sex chromosome abnormalities (SCA) are the most frequently occuring chromosomal abnormalities both at prenatal diagnosis and at birth. Approximately 1/400 newborns has SCA and incidence at prenatal diagnosis is even greater (1/250-1/300).

Among SCA, triple X diagnosis, which has probably little clinical consequences to the affected individual, requires complex and challenging genetic counselling with not fully documented outcome.

We have identified 26 couples who requested a genetic counselling after the prenatal diagnosis of 47,XXX in the first or second trimester of pregnancy (period 1998-2005).

Among these, 7 couples asked for termination of pregnancy, 3 were lost at follow-up, 16 accepted to be included in our study. The protocol included clinical genetic evaluation with auxological measurements and detailed personal history. With parent's consent, pictures of the child were taken to document dysmorphisms and familial traits.

A questionnaire including an assessment of motor, language, behavioral and cognitive skills was then administered. Information about the relationship with the pedaitrician and other family members were collected as well.

Preliminary results did not show any relevant physical or congnitive delay. In three cases language delay was identified. A general high level of anxiety in the relationship between parents and daughter was observed. This observation prompted us to apply evaluation instruments specific for language area and, for age four and above, a Separation Anxiety Test (SAT) for both children and parents.

We believe that this instrument will help us to better undersand the long term consequences of the prenatal communication of a 47,XXX karyotype.

EP28. Development of the genetic counsellor workforce- a UK Department of Health funded scheme

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The 2003 Government White Paper (Our Inheritance, Our Future) on genetics stimulated the expansion of specialist genetic services in the United Kingdom (UK). The government was committed to increasing the genetic counsellor (GC) workforce to help address pressure on genetic services. Most UK genetic counsellors have a background in nursing, but an increasing proportion have completed a Master's degree in Genetic Counselling. This diversity of background was considered a strength within genetics teams and the Association of Nurses and Genetic Counsellors Registration system was designed to ensure that people from a wide range of backgrounds were eligible to undertake the two-year training period prior to registration. However, there was a danger that the need to leave salaried posts to undertake genetic counsellor training would act as a disincentive to qualified health professionals joining the profession.

The AGNC proposed a centrally-funded GC Training Post Scheme to the Department of Health to provide salaried posts for those entering the profession. Funding for up to 50 posts was granted, with financial support for the trainee's salary and educational allowance and a stipend to the host department. On completing training, the post-holder is expected to be ready to apply for registration. Fifteen Genetic Centres in the UK were approved as training centres and, initially, 22 trainees were appointed. Thirteen have now completed their training and eight are near completion. A second phase of trainee appointments has been allocated. Data on the backgrounds, destination after training and the registration status of post-holders will be presented.

EP29. Awareness on Genetics and Genetic Counseling: overview of an outreach programme model for a developing country.

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Low levels of awareness on Genetics, illiteracy, resource-constraints, lack of services near the place of residence, compulsions of work, travel constraints are some of the reasons for people not availing Genetic counseling in the rural, semi urban and other districts of Andhra Pradesh, (India). Most of the people living in such areas have never availed counseling despite the prevalence of Genetic disorders in their families or being affected by them. A model of service to reach this unreached section of people has been evolved and implemented for the past few years. The model involves intensive networking and establishing effective linkages between the professional team offering Genetic services and the medical practitioners, other clinical service providers and the community. The programme serves as a single window system of service delivery to the community at the community itself through community mobilization. The programme comprises of various Awareness building measures, sensitization processes, linkage building, Genetic Counseling, Sample generation and follow up. The model has been a viable approach to offer services to patients and families who have hitherto not availed any professional help for Genetic awareness, counseling or testing facilities. Though patients turn out in large numbers, their needs are identified and met in a very professional manner with due importance to ethical issues.

The paper will provide an overview of such community level initiatives in different places and share the professional, social and psychological impact of 75 such programmes.

EP30. Risk perception, worry and satisfaction related to a genetic counselling session

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Genetic counselling for hereditary breast / ovarian cancer and colorectal cancer was evaluated by assessing patients' perceived risk of developing cancer before and after genetic counselling. In addition, the level of worry and satisfaction with genetic counselling (SCS) were analysed.

Two hundred and thirteen patients with a family history of breast / ovarian cancer or colorectal cancer answered a questionnaire immediately before and after genetic counselling (response rate: 77%).

Results showed that the patients' perceived risk decreased significantly ($p < .0001$) after genetic counselling. However, there was a discrepancy between risk perception in percentage and risk expressed in words. Forty-one percent of the patients believed they had the same or less risk for developing cancer compared to other persons at same age and gender. Furthermore, patients were significantly less worried after the genetic counselling session ($p< .0001$). Low satisfaction with the counsellor, high perceived risk for developing cancer and younger age were predictors of high level of worry. Although the patients were exceedingly satisfied with the genetic counselling (mean score on the subscales of SCS: 11.2, range: 3-12), 27% gave a wrong answer regarding whether they were included in a surveillance program. In conclusion, counsellors seem to met the patients' psychological requirements, but perhaps not their informative needs. More than 40% of the patients assessed their own risk to be the same or less compared to others, suggesting that the patients have difficulties understanding their risk of developing cancer.

EP31. Testing the children: Do non-genetic health-care providers differ in their decision to advice genetic pre-symptomatic testing on minors? A study in five countries in the EU.

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Introduction: Within Europe many guidelines exist on genetic testing on minors, but there is no common EU policy regarding this topic. Genetic testing in children is recommended only under circumstances where a clear medical and psychological benefit to the child can be demonstrated. Pre-symptomatic genetic testing in minors is recommended for disorders for which medical intervention exists, and for which early detection improves future medical health. However, in daily health-care practice it may be difficult to apply these guidelines.

Aim: The aim of this study is to see whether non-genetic health-care providers in five different EU-countries would act similar once confronted with parents request of pre-symptomatic testing on their minor children for a treatable disease.

Methods: Structured questionnaires were sent out in five different EU countries (Germany, France, Sweden, The UK and The Netherlands) to GPs and paediatricians, presenting them with an imaginary scenario, in addition of which questions were asked about whether or not they felt that the children presented in the scenario should undergo pre-symptomatic genetic testing.

Results: There was agreement between countries on testing the oldest child, aged 12, and not testing the youngest child, Tom (6 months). But there were large differences between countries in recommending a genetic test for the child at the age of 8 years. Physicians in France and Germany would recommend a test, whereas physicians in The UK, Sweden and The Netherlands would not.

Conclusion: It is important to formulate a standard non-ambiguous guideline on genetic testing on minors in the EU.

EP32. Written information of MSI-results - how well are they understood?

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Purpose Screening for MSI out of tumour tissue has proven a sensitive procedure to detect possible cases of HNPCC. Within the German HNPCC consortium MSI analysis is performed routinely, followed by search for mutation with a MSI-H result. Subsequent to comprehensive multidisciplinary counselling, participants receive a written summary of the MSI result. So far, no information is available as to how this information is understood and adequately interpreted by counselees. This study aims to explore the consultands' comprehension of a MSI summary letter. **Methods**: 4 weeks after notification of the MSI result by letter, 225 subjects completed questionnaires, aiming to explore the comprehension of results. 107 subjects received a MSS result, 88 were informed of a MSI-H result. In 30 subjects, the result was inconclusive, due to a MSS result in the presence of clinical criteria indicating HNPCC. **Results:** Overall, 90% of consultands considered the written MSI summary comprehensible and easy to understand. 83% of counselees assigned a low risk (MSS) correspondingly rated their risk of HNPCC to be low. Among counselees assigned a high-risk (MSI-H), 52% accordingly rated their risk to be high (correspondence 52%). Among subjects receiving an inconclusive result correspondence of self-rated and assigned risk was found as low as 30%. **Conclusion**: In case of a MSS-result written information appears an appropriate means. In case of MSI-H and inconclusive MSI-results, written information does not ascertain that results are interpreted as intended. Especially for inconclusive results, in-person disclosure and discussion of the consequences is required.

EP33. Psychosocial aspects of genetic testing in families at high risk of developing multiple tumors at various sites and ages.

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Background: Li-Fraumeni Syndrome *(LFS)* and Von Hippel-Lindau Disease *(VHL)* are characterized by an increased risk of developing multiple tumors at various sites and ages for which preventive and treatment options are limited.

Purpose: To investigate the uptake of genetic testing for LFS and VHL, evaluate the psychosocial consequences of *(not)* undergoing genetic testing, and assess compliance with recommended surveillance programs.

Patients and Methods: In collaboration with the nine family cancer clinics in the Netherlands a nationwide cross-sectional study is being performed. The 10 known mutation families with LFS *(± 70 adults and 50 partners)* and the 40 known mutation families with VHL *(± 160 adults and 110 partners*) are invited to participate in the study. Data are collected via postal questionnaires which asses experiences with genetic testing and associated preventive health behavior, cancer worries, general and cancer-specific distress, identity development, self-esteem, family relationships and communication, feelings of quilt, quality of life and attitudes toward prenatal diagnosis and pre-implementation genetic diagnosis *(PGD)*. For a subgroup of participants *(n=100)* a semi-structured interview will address the more complex issues. Data collection has just started.

Relevance: Insight in the impact of genetic testing for family members at high risk of developing multiple tumors at various sites and ages will aid in planning appropriate genetic, medical (i.e., screening), and psychosocial health care services. It will also serve as a model for investigating the impact of genetic testing for other comparable cancer syndromes that undoubtedly will be identified with increasing frequency in the future.

EP34. Other factors of psychosocial impact of presymptomatic testing for late-onset neurological disorders

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The implications of predictive testing for the partner have received little attention so far, as have the psychological implications of presymptomatic testing in relation to family dynamics. What happens in Portuguese and Cuban families affected with late-onset hereditary diseases? What is the impact in these families due to the fact of having relatives at risk or having affected members? Do descendents at-risk with a result of "carrier", really experience psychological well-being similar or even better that the general population? Or does this mean that the expression of this emotional suffering finds its way through somatization? A real psychosocial orientation that takes into account the family dimension, together with the individual one, still needs further and greater research, so does the impact of the diagnosis on the family. Motivated by these psychological aspects, we are carrying out a protocol of research to assess the psychosocial impact and the family functioning of children at-risk inside our predictive programs in Cuba and in Portugal. We present now the preliminary results of 27 Portuguese subjects involved in this research until the present, at a time when their 3 month follow-up has been completed. Until now, no significant differences have been shown among the pre-test and post-test levels of anxiety, depression and somatization regardless of the presymptomatic results. This study is continuing at the present moment, searching for other factors of psychosocial impact of presymptomatic testing and family dynamics.

EP35. Disseminating *BRCA2* test results identified in the research context to relatives of deceased prostate cancer patients: a qualitative study of relatives' experiences.

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This study was established as an adjunct to an earlier, national study at the Royal Marsden Hospital and UK Institute of Cancer Research, which detected pathogenic *BRCA2* mutations in a number of men diagnosed with prostate cancer before the age of 55 (Edwards et al, 2003). The men had died before the results of that study were available, and the Clinical Genetics team at the ICR/RMH attempted to contact the next-of-kin offering an information/counselling session.

The current study is a psychosocial evaluation of the impact of being contacted about the existence of a genetic fault in a deceased relative. A snowball sampling strategy has been used to recruit relatives with whom the next-of-kin has shared this information. We are exploring:

• relatives' reactions to learning about the genetic test results in their deceased relative,

• prior and current perceptions of risk, and risk management decisions

• communication with other relatives

• information and support needs

• whether relatives perceive that they have experienced benefit or harm as a result.

Participants, some of whom have and some have not elected to have genetic counselling, include partners, adult children and siblings of the deceased men. Semi-structured, in-depth interviews with twelve relatives are currently being analysed using a grounded theory approach. Findings will be discussed, including the importance of the role of the communicator who may not be the closest relative. Effective communication and subsequent handling of the information, whether or not this includes engagement, is dependent on a positive relationship.

EP36. Six years follow-up in the psychological protocol for presymptomatic testing for FAP-I (ATTRV30M), MJD and HD: some preliminary results

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Over the past 6 years, we have followed a large number of individuals at risk for three late-onset neurological disorders: Machado-Joseph disease (MJD or SCA3), familial amyloid polyneuropathy (FAP) type I - ATTRV30M, and Huntington disease. They came for presymptomatic testing and entered the psychological follow-up protocol at our predictive and preventive genetics centre.

This preliminary study presents an initial approach to the psychological consequences of presymptomatic testing for those diseases, in a sample of more than 350 at risk persons (60% females, 40% males), which uptook genetic counselling and testing, and underwent psychosocial evaluation.

As indicators of their emotional state, we chose scores of depression and anxiety, reached with application of two psychological scales (Beck's Inventory of Depression and Zung's Anxiety Scale). These have been evaluated at four moments of the protocol: baseline (before testing); and three weeks, six months and one year after disclosure of the genetic test results.

Results shown that, for the vast majority of subjects, genetic testing did not modify their psychological status, into depression or anxiety, and that the average scores did not increase after test result was revealed. In fact, significant changes into a depressive or anxiety disorder have never been identified at any moment, with the use of the adopted instruments; the average scores were lower than the threshold 9 for depression or than 40 for anxiety.

Despite these results are concordant with previous studies in literature, we may still need further research, and implement the use of different instruments and variables.

EP37. Short-term psychological impact of predictive testing for Machado-Joseph disease in the Azores Islands (Portugal)

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Objective: The short-term impact of the pre-symptomatic genetic test (PT) for Machado-Joseph disease (MJD) in the Azores (Portuguese Islands) was assessed in 46 individuals at risk who completed the PT Program. Methods: Scores for depression and anxiety were used as indicators of the subjects' emotional status immediately before the PT and 1 year after disclosure of the results. Results: Global levels of participation in the Azorean PT Program for MJD were high (20.7%), particularly in Flores Island (35.8%). For the total sample, mean scores of depression and anxiety before and after the PT presented without clinical significance. No differences were found for depression and anxiety scores before and after the PT. Furthermore, when grouped by test results (carriers/non-carriers), there were no differences between pre- and post-test levels. Conclusions: Results indicate that the test result did not cause a decrease in the psychological well-being of the individuals tested. The high number of participants performing the PT in the small and isolated community of Flores Island, where MJD represents a source of stigma, was interpreted as an indication that in this particular population the PT offers the individuals at risk the possibility of liberating from a stigma, and, hence, from exclusion.

EP38. Correlates of presymptomatic testing acceptance in late-onset neurological diseases and familial cancers: some preliminary findings within a comprehensive psychological framework

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Having as a starting point the proposal of a comprehensive psychological framework, we developed this exploratory study in order to investigate the relationship between selected psychosocial variables and acceptance of presymptomatic testing.

All participants were persons at-risk, tested for familial amyloid polyneuropathy type I or ATTRV30M (FAP-I, n=80), Huntington disease (HD, n=15), Machado-Joseph disease (MJD, n=17), hereditary breast and ovarian cancer (HBOC, n=18) and hereditary non-polyposis colorectal cancer (HNPCC, n=19). All were interviewed before disclosure of the test results.

Participants who had reported higher acceptance of presymptomatic testing believed that test uptaking had more advantages than disadvantages, relied more heavily on behavioural (problem solving) or cognitive (logical analysis) approach coping-strategies, and had more positive attitudes towards doctors and medicine. On the other hand, participants who had reported lower acceptance of presymptomatic testing showed greater decisional conflict: they were more uncertain about their decision to be tested and felt uninformed, unclear about values (pros and cons of each option), unsupported in decision making and unsatisfied with their decision; moreover, they perceived more disadvantages in presymptomatic testing, and scored higher on neuroticism.

These results suggest that tested individuals may represent a very heterogeneous group, at the affective, cognitive and motivational dimensions. To the extent we can delineate the role of these dimensions in the uptake of presymptomatic testing, we may target pre-test psychological evaluation and genetic counselling more effectively.

EP39. Presymptomatic testing for late-onset neurological diseases and familial cancers: an exploratory psychosocial study conducted in Portugal

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This exploratory study was carried out to analyze differences in socio-demographic and psychological variables among individuals at-risk for late-onset neurological diseases and familial cancers.

All participants were persons at-risk, tested for familial amyloid polyneuropathy type I or ATTRV30M (FAP-I, n=80), Huntington disease (HD, n=15), Machado-Joseph disease (MJD, n=17), hereditary breast and ovarian cancer (HBOC, n=18) and hereditary non-polyposis colorectal cancer (HNPCC, n=19). All were interviewed before disclosure of the test results.

Individuals at-risk for HBOC were older, more educated and considered themselves to be at a higher genetic risk than those at-risk for the other diseases. Subjects at-risk for FAP-I reported significantly higher satisfaction with life than those at-risk for MJD. At-risk individuals for HNPCC reported significantly higher levels of neuroticism than the other groups, and perceived a higher emotional burden than those at-risk for FAP-I. Moreover, individuals at-risk for MJD showed significantly lower adherence to presymptomatic testing than the other groups.Given that FAP-I is a treatable condition and MJD is not, it is not surprising that the former reported higher subjective well-being. It may be that those undergoing HNPCC testing are a less selected and, therefore, a less psychologically robust group than the other groups. Acknowledgement of these differences can be applied to improve psychological evaluation and genetic counselling of at-risk individuals who participate in presymptomatic testing programs. Pre-test assessment of psychological well-being and personality could be used as a basis for targeting those most likely to experience adverse reactions to the test outcome.

EP40. Beyond consent: ordinary abuses in HD presymptomatic genetic testing in Italy.

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HD has been one of the first and most influential experiences of genetic testing. Although the procedure does not offer specific clinical benefits, genetic testing has been implemented as a mean to promote at risk individuals' autonomy and emotional well-being. In order to pursue this aim, the International Guidelines recommend HD genetic testing being managed in a protocol including the provision of pre and post-test counselling in order to reduce the risks of catastrophic reaction to a positive result.

However, during the last years we have seen a diffuse trend to perform genetic test for HD as a "normal" laboratory analysis taking blood samples from people at risk without any specific counselling and we were able to collect and document some of these "abuses". This practice fails to protect people at risk as it does not provide them with the opportunity to evaluate the pros and cons of knowing own genetic condition and to give a really informed consent to the test. Our data suggest that most of these tests have been performed in order to obtain blood samples for research purposes. We propose to assess if similar events have occurred in other countries and analyse how the internationally agreed protocol can assume a normative value for HD genetic testing.

EP41. Psychological evaluation for predictive testing for Huntington Disease and Familial Amyloidotic Polyneuropathy Type1: A Portuguese experience.

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Presymptomatic testing for Huntington's Disease (HD) and Familial Amyloidotic Polyneuropathy Type 1 (FAP) is available at Genetics' Department of HSM, since the late nineties. Nevertheless, it was only in 2005 (upon definitive publication of the new Portuguese law on genetic testing) that we begun following a structured protocol which includes the psychological evaluation of all at-risk individuals.

Although both HD and FAP are late-onset neurological diseases, psychological disorders/psychiatric complaints are exclusive to HD patients, in whom they may represent the first disease symptoms.

The purpose of this work is to characterise the psychological profile of healthy at-risk subjects and to examine any differences between HD and FAP groups. These issues are particularly important when anticipating the impact of predictive results, and instrumental when developing an adequate early intervention psychological program.

8 HD and 19 FAP subjects, were evaluated so far.Besides an interview, we tested each individual using: Acceptance of the Predictive Testing Scale, State-Trait Anxiety Inventory (STAI), MMPI (revised), Risk Perception of being a carrier.

We have found no statistical differences between HD and FAP groups for demographic data, test acceptance, State and Trait anxiety, risk perception, and personality traits. We found a **negative correlation** between **State-Anxiety** and a**cceptance of testing**, and a **positive correlation** between **State-Anxiety** and **childless,** in both groups.

This is a preliminary analysis with a limited number of subjects. However, we will be will evaluating quite some more individuals before EMPAG. Our

experience reinforces the importance of targeted psychological intervention in the context of presymptomatic testing.

EP42. Psychological particularities and personal representations of subjects undergoing to genetic testing for hereditary colonic cancer

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Familial history of cancer could lead to psychological vulnerability that could interfere with genetic counselling and adjustment after result announcement. Our aim is first to evaluate psychological vulnerability of 127 subjects (61 unaffected, 66 affected) who have undergone mutation searching for colonic cancer and secondly to describe personal representations and beliefs, prior to the first counselling.

The questionnaires Center for Epidemiological Scale for Depression (CES-D) and the State-Trait Anxiety Inventory (STAI) were employed to assess distress and open-ended questions were proposed to access to beliefs about genetic predisposition.

RESULTS: subjects who have undergone genetic testing were more depressed than non clinical population (χ^2 =77.84; p=.000). Compared with unaffected individuals, affected subjects were more depressed (t=-2.05;p=.04), more anxious (t=-2.89;p=.005) but were not concerned by trait anxiety. Surprisingly, 30.7% had estimated their own risk as null or weak and 69.3% had considered they were at high or higher risk. Half of the subjects had a bias perception of their own genetic susceptibility, which they defined as higher like others family members, invoking 1) physical and/or psychological similarity with an affected close relative, 2) the number of cancer , 3) or depressed cognitions like fatalism and hopelessness.

CONCLUSIONS: These results highlight the particular vulnerability of subjects participating to genetic testing for colonic cancer. Some subjects have biases, naives representations which could be a barrier to medical information, preventive strategies and emotional well being, following the genetic results. Implications and consequences for future adjustment are discussed.

EP43. Partners of mutation-carriers for Huntington's disease: forgotten persons?

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This study focuses on psychological distress and coping strategies in partners of tested persons five years after predictive testing for Huntington's disease. Sixteen carrier-couples and 17 non-carrier-couples participated in the study. Self-report questionnaires were used, assessing depression level, anxiety, intrusive and avoidance thoughts and coping strategies.

Results: Partners of carriers have as much distress as carriers, and for some distress variables even more (p<0.05 to 0.001). They clearly experience more psychological distress than non-carriers' partners, as expected (p<0.05 to 0.001). Regarding coping strategies, carriers' partners adopt more passive strategies (passive-regressive and avoiding reactions; p<0.05) and less active strategies (social support seeking and problem solving; p<0.05 to 0.001), compared to carriers. For both carriers and partners, the adoption of more passive strategies was associated with more distress and the use of more active strategies with less distress (for carriers: p<0.05 to 0.001; for partners: p<0.05). The presence of children before predictive testing was an additional result-specific distress factor.
Conclusion: Carriers' partners have at least as much psychological distress as carriers, but partners have the tendency to draw back. The results suggest that the grief of carriers' partners may be "disenfranchised", or not socially recognised, as if they have no right to mourn. We moreover interpreted the results referring to psychological defences and imbalanced partner relationship. Finally, we formulate some implications for genetic counselling.

EP44. What representations lie behind presymptomatic testing? A psychodynamic perspective of a case study.

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The aim of this presentation is to think about psychodynamic concepts behind genetic counselling for presymptomatic testing.
We all went through an identification process with the primary objects - mother/father - to construct our own identity. The individuals that approach genetic services are no different. We will try to understand how the process of identification with a sick caregiver can interfere with the subject's thoughts and behaviour concerning presymptomatic testing. As an example of the impact of *identification by introjection*, we will discuss the case of a woman with a difficult relationship with her sick mother, who believed to be positive for Machado-Joseph Disease, even though she had not been tested yet. We will see that this strong belief is the result of a fusion of her own *Self* with her mother's *Self*.
We propose that the representations of at-risk individuals about their sick caregivers and the quality of their relationships (secure/insecure) with them are important issues to consider and a key to understanding their attitude towards presymptomatic testing.
The psychologist must be aware of these representations of the *Self* and realise that they are unique for each case. This is also the reason why an interview can be the most important instrument in the psychological evaluation.
Key - words: presymptomatic testing, mechanisms of identification, secure/insecure relationships, *Self*.

EP45. Illness and symptom causal attributions in single gene and multiple gene conditions having an ambiguous and environmental trigger.

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The study explores the extent to which peoples' attributions reflects the genetic status of their condition and current scientific knowledge.
Types of attributions made by patients (and their carers) with single gene (Thalassaemia, Becker`s Muscular Dystrophy patients, N=38, [TM]), multiple gene having an ambiguous (Coeliac , Psoriasis, N= 77, [CP]) or environmental trigger (Hay fever, N= 77, [HF]) were compared. Participants were interviewed to assess illness and symptom causal attributions. Following factor analysis, *Environmental, Psychosocial, Life-Style* factors for Patients and Carers and *Mystical* (Patient group only), were identified for Disease. For Symptoms *Environmental, Psychosocial* factors for carers and patients and a Mystical *(*patient group) and Life-style factor (carers only) were identified
HF individuals cited the greatest number of attributes for disease and symptoms followed by CP patients. For cause of disease, *environmental* and *lifestyle* factors were cited significantly more by HF individuals, *mystical* factors by TM patients and *Psychosocial* factors by HF and CP patients. Carers of HF attributed the condition significantly more to *environmental* causes, whilst those of CP and HF cited *psychosocial* factors more. For symptoms, *environmental* and *mystical* causes for HF individuals and *psychosocial* attributes for CP patients were cited significantly more. Carers of HF attributed to *environmental* causes and those for CP cited *psychosocial* and *lifestyle* factors significantly more.
Types of attributions made reflect current scientific knowledge - with HF individuals considering *environmental* factors as significant contributors to disease and symptoms, CP individuals emphasising mainly on Psychosocial attributes whilst single gene conditions attributing to mystical factors.

EP46. Qualitative assessment of illness representations in relatives at-risk for late-onset neurological disorders: a grounded theory approach

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This research evaluated the illness representations of persons at risk for late-onset neurological disorders (Huntington disease, Machado-Joseph disease and familial amyloid polyneuropathy - type I ATTRV30M), based on their answers to three open questions.
Our aim was to explore these illness representations and compare them with those of persons at-risk for another genetic disease (haemochromatosis was chosen as the "control" disease, due to the fact that the burden is much less, as it has effective preventive measures).
We presumed that individuals at-risk who come for pre-symptomatic testing of late-onset disorders might present negative illness representations, once they are pressed by the doubt of being or not being a 'carrier' for such incapacitating and incurable diseases.
These questions were subordinated to two themes: (1) illness representation and (2) motivation to undergo presymptomatic testing. (1) Referring to illness representation, questions were: 1- What does this illness means to you?; and 2- What do you know about this illness?; and (2) referring to the motivation the question was: 3- Why did you decide to come for presymptomatic testing?
Using the program NUDIST, we could find several thematic-answers in all questions, and for all diseases, what allowed us to create main categories (thematic-categories) that would then unfold in several other subcategories.
We conclude that quantitative answers may replace representation and expression of feelings and affections. Thus, these individuals have the reason to come for presymptomatic testing outside themselves (in the others), and not inside themselves (in their future or in their need to know).

EP47. Genetic counseling to people with deep-rooted Belief Systems: The Indian perspective.

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India is a Kaleidoscope of diverse cultures, languages, faiths, beliefs, socio-religious customs and sentiments. The population explosion, consanguineous marriages in some parts of the country, marriages between people of the same community enhance the risk for genetic disorders, There remains disparity in levels of economic, social, educational and technological development between the urban and rural population. Dichotomy in Gender roles, lay beliefs on traditional healing practices, the social stigma attached to some genetic conditions and the strong belief in "Karma" - fruit of ones action - also influence the attitude towards resorting to Genetic counseling or genetic tests. In this socio-economic-cultural backdrop, another major factor to be encountered is the lack of awareness on different aspects of human genetics in all strata of the society.
The paper will focus on the challenges and accomplishments in the field of genetic counseling with reference to the Indian context. Comprehensive analysis of more than 3000 counseling sessions will be presented. It will share some field-based experiences and some good practices in the Indian context to reach people with appropriate information on Genetic disorders and the process of managing and preventing them. It will also highlight the changing scenario, efforts to bring the science of genetics to people and the promises it holds for improvement in the situation.

EP48. Experiences of the Clinical Genetics Staff, in Cyprus, about caring for linguistically diverse patients.

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Several cultural components, one of which is language, have a significant impact on the provision of, and access to, health care. There is an increasing need for health care professionals to become culturally competent when they care for patients who are culturally and linguistically diverse. There is a well-established clinical genetic service in Cyprus which provides care for the whole population. Cyprus has substantial immigrant communities. This study aimed to explore the experiences and perceptions of the Clinical Genetics Staff in Cyprus caring for linguistically diverse patients. Semi-structured interviews with 3 staff members of the Genetics Clinic, 1 interning student and 1 multidisciplinary team member were carried out.

All the interviewees were either bilingual (in Greek and English) or multilingual (in Greek, English, French, and Arabic). The Clinical Genetics Staff do not have access to trained interpreters. Patients who speak neither Greek nor English have to rely on other family members, friends or the multilingual clinic staff to interpret and translate for them. All the clinical staff were concerned that not being able to communicate directly with these patients could result in problems of miscommunication, inaccurate translation and risk of interpreters avoiding difficult issues or expressing their own feelings and views. All the interviewees felt the counselling element of consultations was the most difficult when there was a language barrier between themselves and the patient.

EP49. Do women seeking genetic counselling for hereditary breast cancer have a pessimistic risk perception?

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PURPOSE: The purpose of the present study was to examine whether women who seek genetic counselling for Hereditary Breast and Ovarian Cancer (HBOC), assess their own risk different when they compare it to either (a) an average woman in the general population, or (b) another woman also seeking genetic counselling. In addition, we explored some medical and psychological factors related to these women's comparative risk assessment.

METHODS: Before counselling, 620 women filled out a first questionnaire assessing their comparative risk estimates.

RESULTS: Results showed that these women consider their risk higher than that of a woman from the general population (i.e., a pessimistic response), but equal to the risk of another counselee. The latter comparative risk measure was also more normally distributed implying that comparing to a similar other person is a more relevant comparison. Women were more pessimistic comparing to another counselee if they had had breast cancer, and more optimistic if they had no first-degree relatives with breast cancer, perceived a low absolute risk, and had stronger dispositional optimistic tendencies.

CONCLUSIONS: Overall, women who sought genetic counselling for breast cancer had unbiased comparative risk perceptions and correctly assessed their own risk status.

EP50. Previous experiences with cancer in the family - impact on health beliefs and distress in persons at risk of HNPCC

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Background: From a clinical perspective it is regarded common sense that prior experiences (PE) with illness and death shape current perceptions of a hereditary disease and one's own risk. The impact of PE on counselees perceptions has been rarely explored in empirical studies. In this study, the impact of PE with cancer was explored in persons at risk of hereditary bowel cancer (HNPCC).

Methods: Using a prospective design and consecutive accrual, 183 unaffected individuals at risk of HNPCC were assessed. PE with regard to cancer in the family of origin were elicited by interview during comprehensive genetic counselling. Perceptions and distress specific to HNPCC were assessed through standardised and study-specific measures (1) before and (2) 8 weeks after genetic counselling. Regression analyses were performed to determine the impact of various characteristics of the illness experience on cognitions and distress specific to HNPCC.

Results: The perception of cancer-related threat was shown to decrease with the number of relatives affected by cancer, and to increase when counselees had experienced the death of close relatives. No direct effect of PE was found on perceptions of risk; while an indirect effect was mediated by cancer-related threat. None of the various characteristics of PE was related to distress specific to HNPCC.

Conclusion: Differential effects of PE were shown to shape counselees' perceptions specific to HNPCC. Since PE are likely to influence counselees' response to clinical risk assignment, they should be taken into account during genetic counselling.

EP51. Genetic discrimination in Australia: The clinical perspective

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Genetic discrimination is defined as the differential treatment, either favourable or unfavourable, of asymptomatic individuals on the basis of their actual or presumed genetic differences. The Genetic Discrimination Project (GDP) in Australia has investigated multiple perspectives regarding the issue and experience of genetic discrimination, including those of consumers, clinicians and genetic counsellors, insurers and employers and members of the legal profession. Clinical geneticists and genetic counsellors are uniquely placed with regard to the concerns of at-risk individuals regarding genetic discrimination and are required to advise them appropriately according to professional and legal standards. This paper reports on data obtained from Australian clinical geneticists and genetic counsellors with regard to the issue of genetic discrimination, its perceived relative importance to clients and its impact upon their clinical practice. Survey and interview data from about 50 clinical genetic service providers and social workers are presented, with all states of Australia represented. Insurance, privacy issues, family dynamics, employment and social stigma were the domains perceived by genetic clinicians, counsellors and social workers to be of most concern to their clients regarding the potential for discrimination. Experiences of unfair treatment reported by clients regarding insurance and employment were more likely to have occurred with genetic testing for Huntington disease and familial cancer. Data regarding the impact and practice response of clinicians and counsellors to the issue of genetic discrimination are presented. The legal aspects and implications of the issue within clinical contexts are discussed.

EP52. Psychopathological profile of children with common dysmorphic syndromes - transformation or transaction throughout adulthood. Prognostic skills of the postnatal genetic counseling

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Testing the cognitive capacity is a modern diagnostic approach for patients with genetic diseases, which are often diagnostic problems due to their variety.

Aim: To study and to define the specific cognitive and behavioral phenotypes of patients with some common DS and their transaction or transformation throughout adulthood. To indicate typical behavior of the families with affected children according to their communicative capabilities and their expectation from the genetic counseling and their interpretation of the genetic risk.

Results and discussion: These syndromes manifest cognitive capacity deviations of different severity. IQ level in an individual patient changes with age. The patients with DS show the most maladaptive behavior. Autism and ADHD are frequently observed in part of the DS. The relationship between intellectual functioning and adaptive skills is in inverse proportion.

The families are major - introverted or extroverted and several

subgroups.
Conclusions: 1.The patients with some dysmorphic syndromes manifest specific psychological profiles of cognitive and behavioral characteristics according to the genotype and the age. 2.They may be successfully used both in support of diagnostic process and as a basis for adequate medical and psychological intervention and counseling of parents 3. Most of the families interpret the genetic risk mainly in the context of their reproductive memory and the wish for healthy children.

EP53. Let us play... and tell, and draw and build ourselves

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Polland's syndrome is a malformative rare disease and AISP is a young association, born in 2002. Since the beginning, AISP has choosen the way of enlarged and growingly communication between professsional careers and patients, to increase therapeutic alleance and the knowledges about the disease. Thanks to this attitude, AISP has created a net in which people can find answers to the most common doubts and questions. The corrispondence sent to the web site has shown as the most meaningful requests are those regarding quality of life, so AISP decided to organize annual plenary meetings, with professional careers, families and patients, in which scientific information takes as much place as educational trainings. During the last meeting, educational trainings were performed, by a team of seven professional educators and two psychologists, in psychomotility, occupational and music therapy, and narration laboratories. At the same time, a team of five, among thoracic, orthopedic, and plastic surgeons, provide patients and families with tecnichal advices. The effectiveness of this "formula" had been evaluated in a plenary discussion at the end of the three- day meeting. Comparing the results obteined working with 168 among patients and parents, aged from few months to 52 years, we saw that this kind of intervention improves quality of life because it allows them to see and to exploit their own personal resources, always existing in spite of every kind of disease. We suggest to repeat our esperience in all the situations of chronical disease.

EP54. Living With Disability: The Impact on Siblings

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Research carried out on the impact of disability on families has primarily focussed on the parents of disabled children. In contrast, this study, carried out as part of an MSc, looks at the experiences of siblings who have brothers or sisters with learning disabilities. This retrospective study aims to illustrate how, if at all, a sibling's disability can affect a person throughout their life.
In-depth qualitative interviews were carried out in the participant's homes, transcribed in full and analysed for emergent themes. Six participants were involved and reported both positive and negative emotions surrounding their sibling's disability.
Participants were able to clearly recall childhood emotions and describe how these changed as they got older. Many of the emotions experienced were limited to childhood whilst others continued into adult life. Participants also reported how their feelings often became more positive towards their disabled sibling as they got older and gained greater understanding.
The results of this study have implications for both parents and health professionals working with disability and highlights the need to provide adequate information and reassurance to such siblings throughout childhood.

EP55. Attitudes and Experiences of Men at Risk for *BRCA1/2* Mutations

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Pathological mutations in the BRCA1 and BRCA2 genes predispose individuals to an increased risk of certain cancers. This risk is much greater for women who face a relatively high chance of developing breast and ovarian cancer over their lifetime. Research investigating the psychosocial implications of being at risk for BRCA1/2 mutations has therefore focused mainly on women to date. The small body of literature exploring the psychosocial impact on men at risk for BRCA1/2 mutations suggests that their primary concern is for their children but relatively little is known about the way these men perceive their personal cancer risks and what their support needs are. The aim in this study was to explore the attitudes and experiences of men at risk for BRCA1/2 in the context of risk perception, genetic testing and support needs. In-depth interviews were conducted with 8 men (5 BRCA1/2 carriers and 3 at 50% carrier risk). Interview data was analysed qualitatively using tools from Grounded Theory. Many of the men, whilst they apparently viewed their personal cancer risk as 'low', described having made modifications to their lifestyle in order to minimise their risk. All but one of the men described experiencing frequent, but not particularly intense, intrusive thoughts with regard to their own or other family members' cancer risks. The nature of these intrusive thoughts and reasons why the men had sought, or declined, genetic testing were explored. The findings, whilst limited in significance, provide a foundation on which to base further qualitative, and quantitative, research.

EP56. Adults with a genetic condition: genetic service expectations

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In Victoria, Australia, a dedicated genetic service for adults is proposed to extend existing pediatric/obstetric and cancer genetic services. In order to ensure client-centered services, this pilot exploratory study sought to identify the genetic service needs and expectations of adults with a genetic condition, as well as their experiences obtaining genetic information. Adult members of support groups in the Genetic Support Network of Victoria were invited to participate in a series of focus groups. These groups were audiotaped and transcribed verbatim. Data was analysed using constant-comparative method proposed by Glasser and Strauss. A total of 21 individuals (10 men and 11 women) aged 22-66 years participated; they were affected by 9 different genetic conditions. Participants expected a genetic service to provide an advocacy role within the health system and to provide a coordinated service with continuity of care. However, the extent to which these expectations were met varied. Participants highlighted the need for different information and support at different stages of their lives and their disorder. The findings suggest there is a need for genetic services provided by a multidisciplinary team and highlight the need to be responsive to the different psychosocial/medical needs of adults who are affected by a genetic condition. The themes emerging from this project serve to inform those responsible for service planning and development, ensuring services best meet the needs of those for whom it is intended.

EP57. Family experiences of X-linked retinitis pigmentosa (RP) and genetic testing: a case description.

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X-linked recessive RP (XLRP) is generally considered a severe form of RP, with males typically experiencing nightblindness and peripheral vision loss in the first decade, progressing to partial or complete blindness by their thirties or forties. The recent characterisation of two XLRP genes now means that genetic testing is becoming an option for families. However, there is very little research into XLRP families' perception of genetic testing or the psychosocial impact of the condition. Using qualitative, semi-structured interviews, this descriptive case study aims to explore the experiences and reflections of one extended XLRP family, some members of which had previously undergone genetic testing. The results presented highlight the psychosocial impact XLRP can have both on an individual and their extended family. Among the most challenging burdens emphasized were the continuous need for readjustment to deteriorating levels of vision, the uncertainty about prognosis and a lack of understanding in the wider community. The

diagnosis evoked very powerful feelings of guilt and resentment within this family; with damaging consequences for family communication and isolation of an affected relative. The experience of linkage analysis underlined how individual family members can operate using different primary defences and coping strategies, and that such differences can be a source of tension and friction. Experiences of carrier testing and presymptomatic testing revealed psychosocial and emotional consequences and emphasized a requirement for careful pre- and post-test counselling. The findings of this study raise implications for both future practice and research.

EP58. Changes in coherence of speech across time in women at increased risk of developing hereditary breast cancer

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Introduction: 357 women at increased risk of developing hereditary breast cancer participated in a psychological study on effects of regular surveillance, using self-report questionnaires around two surveillance appointments. However, self-reports may reflect opposite conditions[1,2]. Coherence of speech, the ability to talk about a subject in a truthful, consistent manner, is used as a measure to identify such opposite conditions. Objectives: to examine changes in coherence over time, and to explore if coherence was correlated with distress.

Methods: three structured interviews were held in a random subgroup of 43 women. Two were held after a surveillance appointment (m1, m3), and one halfway two appointments (m2). Raters gave the interviews a numerical (1-9; ≥ 5 = coherent) and a categorical score (free, entangled or dismissive). Self-reported psychic distress was measured with the Hospital Anxiety and Depression Scale and the Impact of Event Scale.

Results: mean levels of coherence were at m1: 4.6; at m2: 4.2; at m3: 4.9 ($p < 0.03$). Intra-individually these scores differed > 2½ points in 28% of the participating women. Percentages of women classified as 'free' were: at m1: 33%; at m3: 29%; at m4: 56%. At m3 (the home-situation), the majority of the women were classified as 'entangled' (63%). Higher levels of coherence of speech (numerical) were correlated with lower levels of intrusion (m1), avoidance (m3, m4) and depression (m3). These results and their implications will be discussed.

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EP59. Hidden Images in the Family Album: using art and poetry to raise awareness of the psychosocial impact of genetic conditions on families.

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One aspect of the role of the genetics health professional is to inform and educate health professionals, families and the general public. This paper will describe an innovative project aimed at enhancing public education about genetics and raising awareness of key psychosocial issues for families and society.

The project team consisted of a genetic counsellor and two artists, one a visual artist and the other a poet. The artists conducted a series of workshops with six people who had experience of a genetic condition in the family. The conditions included Down syndrome, Huntington disease, familial cancer and skeletal dysplasia. Following the workshops and discussion with the genetic counsellor on the nature of her work, the artists created an exhibition of work using multiple photographic images, an installation focussing on the work of Mendel, and poetry to convey the complexity of experience and emotion associated with genetic disorders. An exhibition was initially held in a Public Library. The images and poetry have also been used in a number of presentations and workshops and are on semi-permanent display in a public hospital corridor.

Evaluation of the project was undertaken via analysis of freehand comments recorded by attendees, interviews with six persons who attended the exhibition and a written questionnaire to stakeholders. Feedback indicated that the exhibition had raised awareness of the scientific and psychosocial aspects of genetics. The greatest impact occurred through the poetry, with many people reporting that they were emotionally moved by the messages conveyed.

EP60. Psychosocial outcomes of bone marrow transplant for MPS I Hurler Disease: Patient self-report of personality and personal adjustment

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Aims: To explore the composite scores, clinical and adaptive scales of the BASC Self Report of Personality (Reynolds and Kamphaus, 1992) in terms of norms for children and young people affected by Mucopolysaccharidosis I Hurler Disease (MPS IH) post-BMT. Particular attention was given to the Personal Adjustment composite of the BASC-SR and to its contributors. *Participants and Method:* Eighteen MPS IH patients post-BMT participated in this investigation, along with their biological mothers. The children's ages ranged from 8 to 25 years. Semi-structured interviews with the children's mothers were utilised, and the children and young people were administered tests of cognitive function and the BASC: Self-Report of Personality. *Results:* Hierarchical multiple regression on the Personal Adjustment composite of the BASC demonstrated that 95% of the variance could be explained ($F = 18.741_{3,2}$, $p = .051$) by child health and disability factors, and factors associated with the mother and the family environment. In terms of the clinical and adaptive scales of the BASC, no overt behavioural difficulties were observed. However, possible trends emerged, which highlighted adjustment difficulties with school and feelings of inadequacy for the 8-11 year age group; and a tendency towards inhibition and withdrawal for the 12 years and over age group. *Conclusion:* The findings illustrate how aspects of parenting and the family, as well as aspects of the MPS disease, require attention when providing support to patients. They also highlight the importance of appropriate, consistent classroom support, and question whether psychosocial support should be considered within the school environment.

EP61. Social characterization of consultands requesting presymptomatic testing and examples of social intervention

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Communication and information are associated with genetic counselling because they are essential to reduce anguish and anxiety in individuals at-risk for late-onset neurological diseases (Huntington disease, Machado-Joseph disease and familial amyloid polyneuropathy, type I - Portuguese, Andrade or ATTRV30M). These incapacitating diseases have several implications at the social and psychological level: structural, cognitive and emotional changes.

In presymptomatic testing, evaluation by the social worker has the aim to assess an adjusted (resilient) or unadjusted answer of the persons at-risk, when informed about their status of being or not a mutant gene 'carrier' for one of these diseases. Resilience is their ability to answer appropriately to difficult situations (biological or social and psychological conditions), using their internal (intra-psychological) and external (social and affective environment) resources. This ability should allow a good adjustment and social insertion.

Our genetic counselling protocol includes a social evaluation of the following factors of risk: (1) factors connected with familial configuration (separation of the couple, longstanding misunderstanding, violence, alcoholism, chronic disease or death of a close relative, etc.); and (2) factors of their social environment (poverty or economical fragility, unemployment, unfavourable housing characteristics, relational isolation, etc.).

After this assessment, a social intervention will take place if the individual at-risk does not have abilities or competences to cope with their reality, which is changed by disclosure of the results (either if carrier or not).

EP62. The psychosocial aspects in Phenylketonuria in Republic of Moldova

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The psychosocial aspects in Phenylketonuria(PKU) are the following: the doctors can't manage to offer enough time to give parents the instructions on the diet, children are with mental retardation, they can't adopt in the society and parents can't work. Earlier 86% of PKU children were with severe mental retardation.
Methods: Association of Rehabilitation of Children with PKU from Moldova organized a Daily Center for supporting the families that have PKU children under their care. This includes:

1. Parents' School (psychological support and legal redress)
2. PKU School (teaching of low Phenylalanine diet)
3. Parents' Club (exchange of their experience)
4. Preparation of brochures, booklets
5. Didactic materials for parents and specialists

Results: 25 families with PKU children (0-21years) received the teaching courses. 13 PKU children whose families started teaching from the children's birth have normal development. They go to general kindergartens with their diet prepared by parents. These parents have an opportunity to work. 3 children who started a new diet at 3 years have easy mental retardation, but they went to general schools. 1 girl (21years)-restarted the diet on new, she became better and she was employed. Other 8 children whose parents didn't keep a steady diet before this education have the improvement of the development in dynamics.
Conclusions: The vision of the society on PKU children can be changed by the Association of Parents and Specialists by creating an Information and Training Center for parents, young families and specialists in various fields (social, pedagogical, psychological, medical...)

EP63. The psychosocial impact of severe and profound hearing loss for people with NF2

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Neurofibromatosis Type 2 (NF2) is an autosomal dominant condition which almost universally results in the development of bilateral vestibular schwannomas (V.S.). Hearing is impacted by both the existence and surgical removal of these V.S. Schwannomas also occur on the other cranial, spinal and peripheral nerves. (Evans et al,2003)
Little is known about the psychosocial impact on affected individuals of living with NF2. A qualitative study was designed to explore the experiences of adjusting to significant hearing loss as a consequence of NF2. Six adults affected with NF2, all of whom had lost their hearing in one ear (n=3) or were completely deaf (n=6), agreed to be interviewed. Participants were given a choice of how they wished to communicate in the interviews, i.e. light writer, lip-reading etc and the approach to conducting research interviews for deafened people with poor morbidity will be discussed.
Interviews were fully transcribed and participant's accounts were analysed for emerging themes using the constant comparison method (Glaser and Strauss, 1967).
Although the study was designed to look at the impact of hearing loss in NF2, all the participants spoke of the combined interaction of hearing loss with the other physical effects of the condition including tinnitus, problems with balance and chronic tiredness. In addition to the physical difficulties, all had experienced emotional and social consequences including feelings of isolation and problems communicating with friends, families and health professionals. The key impacts of living with NF2 and how participants felt these could be helped will be presented.

EP64. Parents and children: transmitting genes, knowledge and responsibility.

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From our corpus of interviews with families about their genetic conditions, we have identified themes that relate to parental decisions about the genetic identity of their children. These include decisions about whether/when to pass on to the child information about the potentially inherited disorder in the family, and the possibility of genetic testing to clarify the genetic status of a child.
Parents may acknowledge that they should discuss their family's genetic condition with their child, and the issue becomes one of finding *the right time*. Parents recount their own experiences of finding out that they had inherited the condition to account for their decisions whether to pass on information to their children. They can draw upon "bad experiences" EITHER to explain why they wish to do better (be more open with their child) OR to justify/explain why they are unable to do this.
Suggesting that a genetic test be performed on a young child can be proposed as a way out of such difficulties EITHER because the child may not have the faulty gene OR because it would ensure that the parent can transmit the genetic test result to the child whether or not the child, when older, would choose to be tested. In this way the parent hopes to pass on to their child their moral responsibility as well as their genes.
We discuss the variety of accounts given by parents of their decisions and what we can learn about how they envisage their child becoming an autonomous individual.

EP65. Attitudes towards genetic diagnosis and coping strategies of persons suffering from hereditary spastic paraplegia

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Hereditary spastic paraplegias (HSP) are a group of late-onset, genetically caused, neurodegenerative disorders. A few can be diagnosed genetically. The aim of the study was to investigate attitudes towards genetic diagnosis and coping strategies of families affected with HSP.
We distributed 410 questionnaires as following:

	Questionnaires delivered	Questionnaires received	Percentage
Patients	307	132	43.0
Patient's partner	38	12	31.6
Risk-persons	48	11	22.9
Risk-person's partner	17	2	11.8
Summary	410	157	38.3

Here, we will report the results in the patients' group only:
102 (77%) of the patients back up genetic diagnosis in order to know their own gene status (58), to prevent gene transmission (19), to plan personal life style (18) and to manage family planning (14). Only six reject genetic diagnosis, because knowledge is insignificant (4), there is neither cure (4) nor prevention (2) of HSP. Psychological problems, problems with social environment and data protection do not play such an important role as we know e.g. from Huntington's families.
Coping strategies were investigated by using the Trier Coping Scales. In summary, patients suffering from HSP cope more in an active manner than in a depressive or avoiding one. Support in coping was more expected from neurologists (78%), family members (66%), GP's (63%) and partners (59%) rather than by self-help groups (33%), genetic counsellors (30%) or psychologists (17%).

The specifics of HSP compared to Huntington's disease or heredoataxias and differences in attitudes and coping strategies will be discussed. The knowledge of these differences may be important for genetic counsellors.

EP66. Exploring causal beliefs, perceived risk and controllability of diabetes type 2 among high-risk individuals with and without a family history of DM2

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A family history of diabetes type 2 (DM2) is one of the strongest risk factors for DM2, reflecting the consequences of genetic predisposition, shared environment, and common behaviour. Despite the prevalence of DM2, little is known about the perceptions of (risk of) DM2 among high-risk populations. Using semi-structured interviews, causal beliefs of DM2, perceived DM2 risk and feelings of controllability were explored among high-risk individuals aged 56-75 years with (n=9) and without (n=14) a family history of DM2.

Most participants perceived DM2 as multifactorial disease, and could often name several causes including 'genetic factors'. Although the majority of people with positive family history was inclined to mention 'genetic predisposition' as a cause, this was not always brought up in explaining why family members were affected. The role of 'genetics' was seen as more pronounced when more than one relative was affected ("it runs in the family"). Overall, DM2 risk was perceived low in both groups. Only four individuals with positive family history perceived a (slightly) higher risk compared to other people of the same age. The absence of DM2 in the family was often used as a reason to perceive a low DM2 risk. Ways to prevent DM2 were mostly unknown or were not seen as very effective, especially when genetic predisposition was seen as major cause for DM2, suggesting a sense of fatalism. This research indicates the need for more public health communications on the role of a positive family history in causing DM2, and on possible preventive measures.

EP67. Determinants of familial risk perception of common diseases

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Background: Taking a family history is increasingly becoming part of the risk assessment and management of common chronic disease in primary care. Patients' understanding of their family history may influence perceptions of both their risk of the disease and its management.

Objective: To identify which factors best predict familial risk perception among primary care patients with a family history of one or more common chronic diseases.

Design & Methods: A survey of UK patients identified in general practice, having a family history of either cancer, heart disease or diabetes. The FRisk questionnaire was developed using both established measures and items drawing on our recent qualitative work.

Results: The 754 respondents (response rate 62.0%), were mainly female (61.1%) white British (92.3%), with mean age 48.6 (SD 11.9). Multiple regression analyses revealed that believing that the disease 'runs in the family', is a serious condition, and has a genetic cause, were the most significant predictors of familial risk perception. Younger age, being of non-white British ethnic origin, feeling 'like' the affected relative, and with vulnerability at a similar age, were also significantly related to higher risk perceptions.

Conclusions: Predictors of familial risk perceptions among a primary care population may differ from the biomedical model of identifying the number of relatives with the disease and age at which they were affected. The findings will inform the development of a family history screening instrument to facilitate both management of familial risk of chronic diseases, and behaviour change to reduce the disease risk.

EP68. Being at risk for a genetic disease, fatalism and the role of the self

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Providing people with genetic risk information may induce preventive behaviour if a person beliefs that this can reduce the risk. However if a person assigns excessive causation to genes, he or she may either adopt a fatalistic attitude towards the risk and/or accept only medical approaches. Perceptions of controllability of genetic risk may be linked with the way people see themselves; the self-concept. People with a static self-concept (SSC) consider fixed traits as primary causes of behaviour and process self-relevant information in a way that is consistent with this deterministic perspective. People with a dynamic self-concept (DSC) understand themselves in terms of goals, needs and states of mind and put more emphasis on situational influences. It is likely, that when faced with a health threat, people with SSC are more susceptible to feelings of fatalism and show less preventive behaviour, especially when the threat is associated with a genetic susceptibility. To test this, a static-dynamic self-concept questionnaire was validated. The final (7 item) questionnaire displayed some predictive validity. When asked to imagine themselves in different health scenarios (representing a lifestyle risk for cardiovascular diseases (CVD), a risk based on a positive family history of CVD, and a genetic risk for CVD), people with SSC compared, to people with DSC, perceived less control and showed a stronger preference for cholesterol-lowering drugs over lifestyle changes, especially when the risk was presented as genetic. The findings suggest that the questionnaire can be used to help explain differences in responses to genetic risk information.

EP69. Professionals' opinions on population cholesterol screening to detect inherited high cholesterol

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To detect Inherited High Cholesterol in an asymptomatic stage, a Dutch patient organisation offered cholesterol tests via mass media invitation to persons with a family history of cardiovascular diseases. If tested positive, persons were referred to health care professionals.

We conducted individual semi-structured interviews with 13 professionals (in the fields of cardiology, genetics, ethics, psychology, justice, policy and patient interests) to explore opinions about the media campaign (including the cholesterol test offer) in population screening. Ethical concepts and WHO criteria for genetic screening were used for analysis.

While almost all professionals classified the cholesterol tests offered as population screening, many did not perceive it as genetic screening, because no DNA was examined (ignoring the Dutch Health Council definition). Four professionals were generally positive about the campaign, as it 'increased people's autonomy and beneficence (i.e., enabled informed choice for preventive measures) and increased justice (i.e., notified all persons irrespective of family communication)'. Two professionals were generally negative, as the campaign 'decreased the autonomy (i.e., by pressure to test and to enrol in follow-up), decreased the perceived importance of environmental risks, and increased medicalisation and blaming-the-victim'. Seven professionals perceived benefits of the campaign, but had as well doubts: insufficient public information on follow-up, insurance discrimination, and lack of adequate care for persons tested positive.

To conclude, the professionals did not speak the same language and did not agree about the acceptability of mass media approaches to detect familial predispositions. A debate among stakeholders may improve communication and forming of more united opinions.

EP70. Risk perception among women receiving genetic counselling: A population-based study

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Background A number of studies have demonstrated that women who

receive genetic counselling for hereditary breast or ovarian cancer improve their perception of risk after counselling. However, a great number of women still over or underestimate their personal risk post-counselling. Most studies on genetic counselling lack control groups, and to our knowledge no studies are population based. It is thus still questionable whether genetic counselling provides a more accurate risk perception when genetic counselling is a standard service offered throughout the population of women at risk of hereditary cancer.

Purpose To examine women's perceived lifetime risk of cancer, the accuracy of risk perception and possible predictors for inaccurate risk perception after counselling.

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 counselling or mammography and 12 months post-counselling. Data on the counselling session, including the assessment of the woman's objective risk of breast cancer, were retrieved by questionnaires from the doctors providing the counselling.

Results Final results of all research questions examined in the study will be presented.

EP71. Exploring patients' views on pharmacogenetic testing

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Clinical evidence indicates that individual people may react differently to similar doses of medicines. Pharmacogenetic testing (PGx) is advocated as an approach to predict how patients will respond to certain medicines. There is no published evidence on patients' views of PGx. It is vital to explore patients' opinions before the widespread introduction of PGx into healthcare practise. This study aimed to explore patients' views on PGx services provided by the British National Health Service (NHS). Semi-structured interviews were conducted with 11 patients (mean age 56 years; 7 women) with inflammatory bowel disease, currently taking medicines. A topic guide was developed using the published literature and show cards with a lay definition of PGx. Interviews were recorded and transcribed verbatim. Data were analysed using the constant comparative method. This presentation will describe the key emerging themes. Some examples of the themes emerging are: patients had no previous experience of PGx and found it difficult to define accurately; PGx was perceived to be of benefit both on a personal level and for the general public; patients gave opinions about PGx services based on their experiences of their illness, taking medicines and using the NHS. This is the first known UK study to explore individual patients' views of PGx services. Although patients were initially unclear about the definition of PGx, they did understand the concept of what a PGx service could offer. Patients gave clear examples of their perceptions about the benefits, costs and the potential implications for developing a healthcare service.

EP72. Public attitudes towards human genome science in Japan_willingness to donate their bloods

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Objectives: While some large-scale and long-term prospective genome studies were launched in Japan, peoples' attitudes toward human genome sciences are unclear. The purpose of this study was to describe their present attitudes, uncertainties and information sources and seek further discussions for better science communication.

Participants and methods: A postal questionnaires to 4,000 adults randomly sampled nationwide. Participants were asked about their scientific literacy, feelings, values and risk cognition toward genome sciences including basic sciences, health related sciences and food sciences.

Results: A total of 2,171 completed the questionnaire (991 men and 1,180 women; 18-60 years old). The response rate was 54.3%. Of 70.5% of participants were "interested" in health related genome studies and 69.6% agreed to promote these studies. With regards to willingness to contribute to research, 39.1% agreed to donate their blood, 13% disagreed and 47.1% were uncertain. Of 39.1% participants, they donate their blood if researchers disclosed "my own" results (78.5%) in safe environment (71.4%), when privacy were strictly protected (69.4%). Half of respondents seek direct communications with researchers; informed consent in plain language (56.5%) and disclosure of interim research results (51.9%). Unwillingness was constructed by unexpected disadvantage (40.7%), possibility of breach of privacy (38.4%), confidentiality of genetic makeup (35.1%) and just scariness (33.6%).

Discussions: In spite of lower willingness rates and accompanying conditions, higher participant rate, 75-85%, is observed in genome studies in Japan. Focus group interview studies will be followed to confirm absence of compulsion or persuasion to participate in their decision making process.